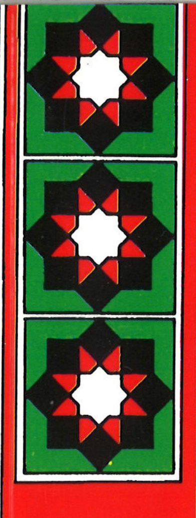
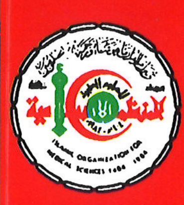
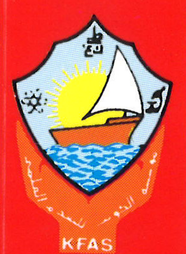


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Islam and Recent Medical Problems





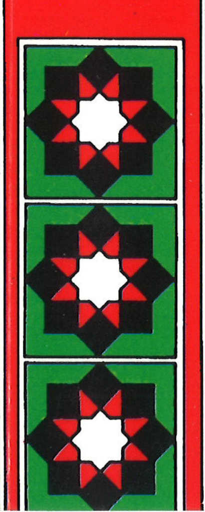
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USED IN ISLAMIC MEDICINE

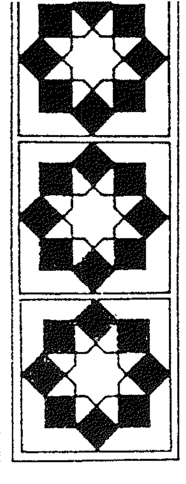
Pharmacological, Toxicological, Biochemical and Therapeutic Evaluation

Supervised by
Dr. Abdul Rahman A.Al-Awadi,
President,
Islamic Organization for Medical Sciences,
Kuwait

Edited by
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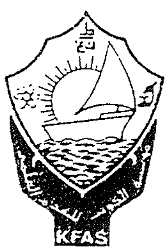


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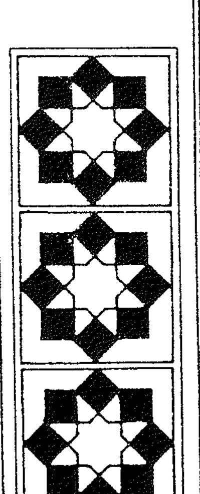
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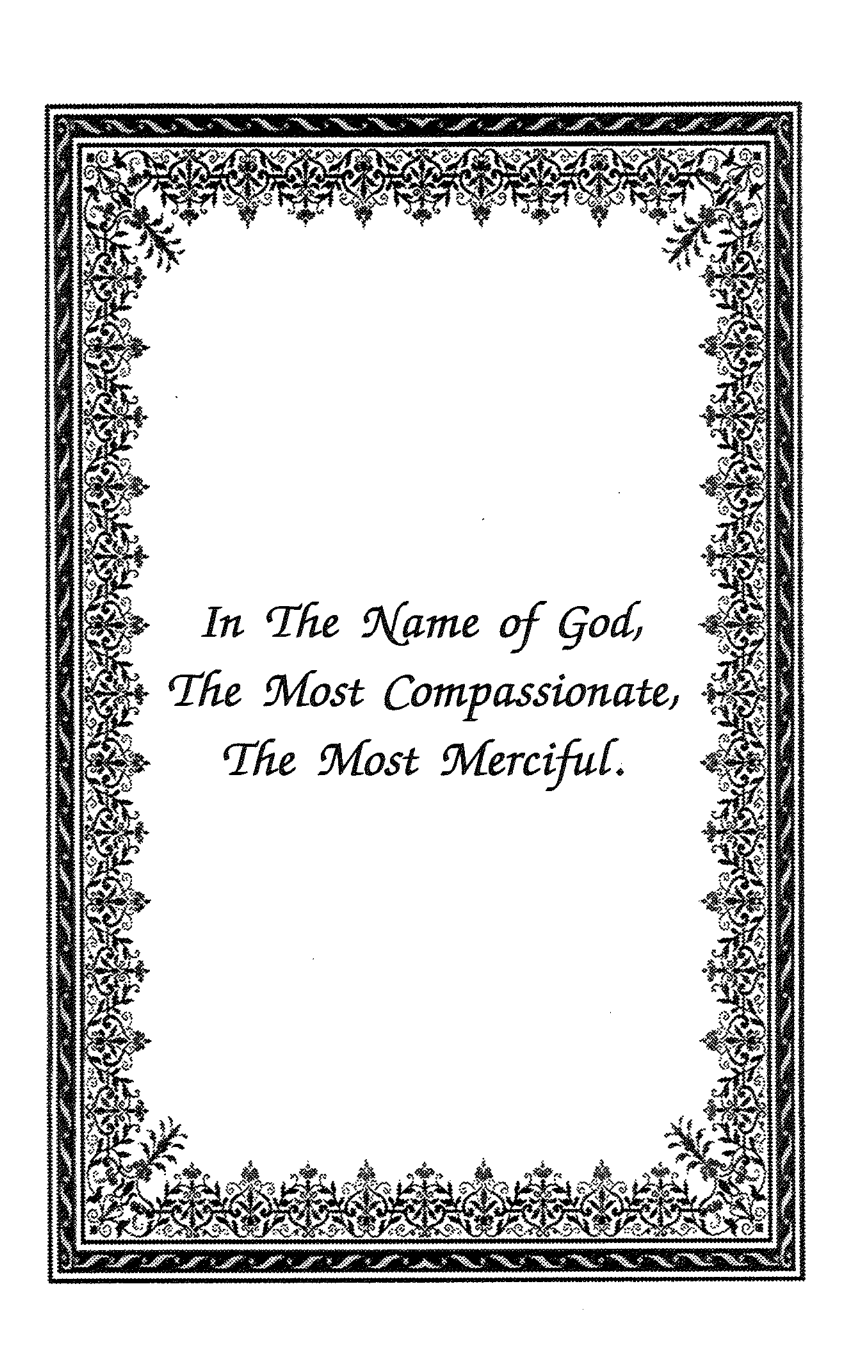
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In The Name of God,
The Most Compassionate,
The Most Merciful.

CONTENTS

PREFACE

Dr. Abdul-Rahman A. Al-Awadi
President
Islamic Organization for Medical Sciences
KUWAIT

Thanks to Allah Almighty for guiding us to Islam, enlightening our hearts with true belief, discarding all grief, dispelled worries, and freed our homeland.

This series comes following fifteen years of the idea of establishing the Islamic Organization for Medical Sciences and after its participation in local and regional book exhibitions where our volumes of Islamic Medicine were greatly appreciated by the visitors. However, because of the soaring cost of paper and publication, the individual book keeping has become very difficult, especially in the non-Gulf Arabic and Islamic countries, as bread earning receives the first priority of the inhabitants of these countries. Keeping in view the fact that the individuals need to be informed, and educated, of the important matter to make them effective member of their community and also a messenger to other communities, it is vital to provide them the contents of these conferences in a simplified way to enable them to carry along and comprehend the scientific purport.

In order to facilitate the possession of these books by the individuals, the Islamic Organization for Medical Sciences has decided to issue a series of publications under the title "The Cultural Series of the Islamic Organization for Medical Sciences". Although the Organization is shouldering the largest share of the cost of production and publication of these books, still these are out of reach of a large section of Muslim individuals, due to escalating cost of living. The great sum of money available to the Organization

is spent in bringing together and collecting the prominent thinkers of our Islamic nation in order to achieve appropriate opinions and covisions of the Islamic Scientists about right topics that need insight and the true objective word. And, subsequently, to present this information to every individual willing to increase his/her knowledge about the doctrinal writings in scientific medicine, as this prominent group of writers/thinkers sees this as an ordinance and a religious obligation to provide for all the Muslims, and to disseminate the message to the largest number of the people of this nation.

This series will include a group of books, each dealing with specific topic, as collected from the articles written under the respective domains and previously published in the Proceedings of the Islamic Medicine Conferences held under the auspices of the Organization. Moreover, all these publications shall remain concerned with one vital topic, that is, the Islamic Medicine. By doing so, we hope to have shouldered the burden off the Arabic/ Islamic reader to enable him/her to own the right material and hoping to have clarified a lot of mystery about the subject of Islamic Medicine to the Muslim and Arab readers.

Herein, I beseech Allah to guide our steps to what He likes and approves of.

INTRODUCTION

Dr. Ahmad Rajai El-Gindy
Secretary General Assistant
Islamic Organization for Medical Sciences,
KUWAIT

Thanks to Allah, the Almighty; the thanks of the grateful, the obedient, and the desirous of His forgiveness and retribution, beseeching him, to guide us to the right deeds, with praying and blessing his illiterate prophet (ﷺ) who said,

> *"When Adam's son dies, everything is separated from him except for three things, a current charitable deed, a righteous boy praying for him, and a useful science."*

We pray to Allah that these series of publications will be of scientific use to the Muslims in particular, and to humanity in general.

This introduction will be included in all the publications of this series in order to acquaint the reader, who wishes to acquire one or more parts of it, with the objectives of the Organization, and the reasons behind its being established. We wanted to put down these words to the readers concerned about what we did, while the second part of this introduction will be specifically written for each book, including a summary of the researches therein.

Since the emergence of the idea of the Islamic Medicine fifteen years ago, the discussion of the meaning of "ISLAMIC MEDICINE" did not stop; the people argued: Is there an "Islamic" and "a non-Islamic" Medicine? and we found ourselves in front of three opinions:-

The first opinion:-Medicine is a human heritage; inherited successively by generations, and it is a human experience, acquired by technical and scientific practice, and religion has no role in it,

and there is no need to indulge Islam in this subject to protect it from human practices.

The second opinion:- Islamic medicine means nothing to them except it is a past heritage, and we do not need it now because the world is talking about organs transplantation, genetic engineering, Lazer beams... etc. They even considered it a call of underdevelopment, and we have to put it behind closed doors; those are who don't want Islam to be mentioned at all.

The third opinion:- Although medicine is human practices and experiences, but every religion and every heavenly message has its own nature, ethics and practices which are derived from its teachings, and which adds to it its own style. The Islamic era was characterized with a comprehensive change in both the concepts and practices of the people; these concepts and practices were derived from the Holy Quran and the honored Sunna, and were followed by the Orthodox Caliphs, which produced a good harvest, with which they ruled the world, east and west with a civilization - Man was its master, good science its way and the strong belief its pillars. This civilization lasted for five complete centuries, and it was never stingy with its knowledge and arts on humanity.

For there is no favor of an Arab on a Persian, nor of a white man on a black man except by piety and good deeds, this was said by the enemies before the friends; and (Sarton's) testimony in his encyclopedia, the history of sciences, is the best evidence; (Sarton) divided the world into eras of civilizations like the Pharonic, the Babylonian, the Somarian, the Chinese, the Greek, then the Islamic Civilization which flourished in all walks of Arts and Sciences for five consecutive centuries, and in it were eminent scientists, thinkers, philosophers, physicians, pharmacists, engineers, Algebra's, Astronomers, Agriculturists, and people of thoughts who were distinguished with their excellence in the Divine Law, besides the cosmetic sciences.

To all these we say, our view of this topic is derived from Islamic Law, which came with its five goals, which are sustaining the religion, the mind, the self, the honor and the wealth. If we studied these goals, we'll find that three of them are concerned with Man's well being; that is the mind, the self and the honor, as for the other two, they are concerned with man's health, as there is no keeping of religion, nor of wealth without a strong good Muslim (The best one to hire is the strong and honorable). The prophet (ﷺ) defined three main points, if provided in any MAN, he will lead a very happy life, as he (ﷺ) says

"The one who sleeps secured in his bed, healthy in body, well provided for his day's food, ... he is like the one who owned the entire world."

In other words, he has got social, health and psychological security. Thus the Islamic Law talks about well being in its widest range. "The strong believer is more loved by Allah than the weak one, and both are good." The Islamic Law did not speak about medicine in its narrow sense, through which the others are trying to attack us, but medicine is the means of health, and Al-Ghazaly, a Muslim religious leader, considered medicine as a religious ordinance in all Muslim homes.

Islam considers enjoying a good health one of the biggest blessings of Allah; as mentioned in the wise saying of the prophet (ﷺ), "*Two blessings many people are not endowed with; health and leisure time*". These two blessings are two of the very important duties that must be kept by man as the Islamic rule says, "Whatever is not perfect without a duty, is itself a duty", thus man is not allowed to neglect his health, as it should not be neglected, because this is considered an aggression on the whole nation as it is so mentioned in the Holy Quran:-

"FOR THAT ACCOUNT WE ORDAINED FOR THE CHILDREN OF ISRAEL THAT IF ANY ONE SLEW A PERSON -

UNLESS IT BE FOR MURDER OR FOR SPREADING MISCHIEF IN THE LAND - IT WOULD BE AS IF HE SLEW THE WHOLE PEOPLE, AND IF ANY ONE SAVED A LIFE, IT WOULD BE AS IF HE SAVED THE LIFE OF THE WHOLE PEOPLE"

(Al-Maeeda: 32).

Abu-Bakr, (رضي الله عنه) said "I heard the prophet of Allah (ﷺ), saying, *"Ask Allah for certainty and health, for they are the best blessings bestowed on man is being healthy after being certain"*; thus self-relief is the true gate to health; either psychological or bodily health, their only true gate is strong belief, belief in slavery to Allah, whatever inflicts you was not to wrong you, and whatever to wrong you, was not to inflict you.

The belief in the acts of worship which are prescribed by Islam are:-

Prayer is secret talk with Allah Almighty, and self purification five times a day standing in front of the Creator,

Fasting is self restrain from evil desires, and true feeling of the hunger of the Muslim brother who is deprived of a morsel of bread,

Zakat or *Alms* is a sacrifice, self cleanliness, and development,

Haj is a migration to Allah and his prophet, (ﷺ), leaving everything - power, wealth, prosperity and living in complete humbleness and slavery, equal with your kin Muslim... as it is said; "No Arabic is better than a non-Arabic, nor a white is better than a black man except by piety", and these acts of worship protects and restrains man from evil doings, thus leaving them will lead to the spread of evil deeds and man will gain nothing but punishment for what he had done.

In order to complete the building of man and society, and to achieve the goals of Islamic Law, the doctrines of lawful and unlawful were put down to guide man to the right road and bestow happiness on him; as the in lawful deeds man will find his happiness, and in unlawful deeds he will be perished; thus the

prohibition of drinking alcoholic drinks, and all ways leading to it, as prescribed by Allah was for the protection of man's mind and body, the society from diseases and the consequences of the absence of his mind, the prohibition of adultery, and all ways leading to it, wanton display of beauty, solitude with a woman, and libertinism... etc, was prescribed to protect the family and the whole society from dissociation and mixing of lineage which destroy the society, thus the philosophy of prohibition in Islam is meant for the prevention of harms to man himself and to others as well.

Thus, it is clear that the goals of Islamic Law (Sharia) can not be achieved without good health and well being, as Abu-Al-Dardaa said to the prophet, (ﷺ), "To be healthy and grateful, is much more better than to be ill and endure patiently", the prophet (ﷺ) answered him by saying, *"Allah loves healthy people, as you do"*.

That is not all, but Islam's view of the sick and sickness has overrun all that preceded it and whatever followed from laws or social systems, as Islam does not see sickness as an anger of Allah, or a touch of the devil, but a trial, and the Muslim has to be patient and bear it with patience as the Prophet (ﷺ) said,

> *"Any kind of sadness or grief or even the prick of a thorn that inflicts man is a blessing from Allah as He raises him a degree higher or takes from his bad deeds instead".*

The Holy Quran came to the world with statements about the inner self, this was fourteen centuries ago, and it put to it four marvelous divisions in various parts of the Holy Quran, thus the world knew about the peaceful innersoul, the lamenting innersoul, and the authoritative innersoul. Abu-Hamid Al-Ghazally, has delved deep in the inner-self in his encyclopedia "The Revival of religious sciences", under the heading" Fear and Request", as the Holy Quran talked about the ailments of the heart, and their different kinds, as it was mentioned by Imam Al-Zahaby in his book "The Prophetic Medicine".

As for the medicine of the heart, it is only found in the sayings of the benevolent and kind Prophet (ﷺ), when he quoted Allah, the only source of all knowledge, he says that for the hearts to be righteous, it must know its creator, His names, characteristics, deeds, orders, and prohibitions and anger, as there is no way of being righteous except by doing this, and no way of getting these advice except from Mohammed (ﷺ).

Imam Ibn Kerium Al-Jozeiah has divided the hearts into two divisions: suspicion and doubt, and desire and error. He quoted the Holy Quran as saying,

"IN THEIR HEARTS IS A DISEASE; AND GOD HAS INCREASED THEIR DISEASE".

(Al-Baqarah: 10), and:

[O CONSORTS OF THE PROPHET! YE ARE NOT LIKE ANY OF THE OTHER WOMEN: IF YE DO FEAR (GOD), BE NOT TOO COMPLAISANT OF SPEECH, LEST ONE IN WHOSE HEART IS A DISEASE SHOULD BE MOVED WITH DESIRE]

(Al-Ahzaab: 32).

The Quran described the inner-self when horrified or frigh-tened, and how to make it peaceful again in His very simple and clear words:

"TRULY MAN WAS CREATED VERY IMPATIENT; FRETFUL WHEN EVIL TOUCHES HIM; AND NIGGARDLY WHEN GOOD REACHES HIM; NOT SO THOSE DEVOTED TO PRAYER: THOSE WHO REMAIN STEADFAST TO THEIR PRAYER; AND THOSE WHOSE WEALTH IS A RECOGNIZED RIGHT FOR THE NEEDY WHO ASKS AND HIM WHO IS DEPRIVED (FOR SOME REASON FROM ASKING) AND THOSE WHO HOLD TO THE TRUTH OF THE DAY OF JUDGMENT; AND THOSE WHO FEAR THE DISPLEASURE OF THEIR LORD, FOR THEIR LORD'S DISPLEASURE IS THE OPPOSITE OF PEACE AND TRANQUILLITY."

[Al-Maarij: 19-28].

This is how Islam considers health, which was defined by the prince of Islamic physicians: Ibn-Sina by saying: "Medicine is the science by which the human body is known, and what is good and what is not for being healthy or otherwise." This comprehensive definition which was introduced more than one thousand years ago, is nowadays adopted by the WHO, that health is the state of the healthy body, mind and society, not only the lack of diseases or inability.

In spite of this definition of the WHO, during the forties, it ignored the spiritual side, which shows the lack of a comprehensive view of Islam about health, as Islam defines health from all domains, bodily, spiritually, psychologically and socially, and this last definition came 14 centuries ago, by the Muslim physicians.

To reach these noble goals, and great objectives for the Lord's heir on earth, there had to be a way to keep man healthy, and this is by the science of medication which was considered by the Muslim religious scientists an ordinance in the Islamic world, and Imam Al-Shafeiy said about it; "There is no knowledge, better than the prohibited, and non-prohibited acts, to my knowledge, except the science of medication". Dawood Al-Antaky in the introduction to his famous prescription says that there is no science that can do without the science of medication, because no acquisition of any knowledge is perfected without a sound body, senses, and mind.

Islam has taken good care of the different branches of medication; protective, preventive, an rehabilitative; in the protective, many sayings of the prophet (ﷺ), called for protection, in order to keep health in all its branches - cleanliness, food organization, and many healthy habits, as well, the researches in this domain is varied and all are derived from the prophet's wise sayings, no need to repeat them here.

As for the treatment side Islam legalized medication, and the prophet (ﷺ) ordered medication and looking for it when he said:

"Ye believers, get treatment, the Lord created no disease without its medicine, known to those who know and ignorance to those who don't know".

As for rehabilitation, we are asked to look for it, he allowed one of his disciples to put a piece of gold on his lost nose during his invasions.

As for the three opinions pre-mentioned concerning the definition:-

To the first group we say: Medication is a human heritage and contribution, but the human thinking has deviated from the right path, and religion is in the church and in the mosque or the temple, due to their sufferings from the control of the church over medication and sciences, and making them only for the priests, medication did not develop, and the ship of science sank deep with its arsenal of destruction, thus they produced the microbial bombs, and medication turned into fatal poison; instead of relieving pains, and becoming a tool of the Lord's benevolence, it became devastatingly harmful, and the brother became keen on eliminating his human brother, and the call for killing substituted the call for mercy, the organs began to be sold, and man was transferred from the master of earth to a sample in labs, and source of trade .... etc. the list is endless.

The best evidence to be quoted here is the saying of Abenhaimar; the father of the atomic bomb, when he saw it explode in Hiroshima from a distance, he said his famous words: "Now, and now only, science has sinned".

As for the second group: which said "Islamic medicine is nothing but an ancient memory and a call for underdevelopment.." we say to them that the heritage of any nation is like the roots of a tree, whenever it goes deeper and deeper in history, it becomes firmer and firmer and provides it with the means of living; the invention of genetic engineering, the nuclear bomb, and organ

transplantation are not only signs of civilization, but they are the leaves of the tree and its fruits, as civilization is much more wider than that, and cares less with its achievements, but cares more for the achiever, MAN, and cares for the philosophy of his existence in this world and the hereafter, as well as his ethics and culture.. if he is separated from these, he will be lost for ever. Now although the western man enjoys the highest per capita, and has got every means of prosperity, we find the percentage of suicide going up and up, as well as the addiction of narcotics, drugs... etc. became a daily practice; to enable him to forget and escape from his worries... the western man neglected the spiritual side of feeding his inner-self, and instead tried to feed on earth's food, thus he failed, and was transferred to a cog in a big machine.

This is not only in the west, but it is now prevalent in the east, as well; family relations are severed, social relations collapsed, man changed into a wild beast in a jungle full of fierce animals, each is trying to eat the other. I don't want to say more, it is enough to remind you with the AIDS that is harvesting man's bodies... Nevertheless, no body talks about chastity, virtue or ethics.. but they began to distribute contraceptives, for males and females, as if saying "Do it however, and whenever you want..! but use these contraceptives to protect you from the AIDS..!" Is this the Islamic way or attitude towards the man, whom it honored and asked to walk and learn and enjoy the fruits of life. Man asks, as many asked before about health and happiness, in spite of his materialistic progress and scientific development in all fields of medicine and protective treatments.

Islam gave due attention to man's environment, and warned him against corruption and doing mischief, as both affect his health, the Lord's words describe what happened all over world from corrupting the environment, which threatens man's life as He said' "CORRUPTION HAS APPEARED ON LAND AND IN

SEA ON THE HANDS OF MAN, TO MAKE HIM TASTE SOME OF HIS DOINGS, HOPING HE MIGHT RETURN TO RIGHTEOUSNESS", and He orders us not to do mischief by saying, "DON'T CORRUPT THE EARTH AFTER IT HAS BEEN RECLAIMED." Corruption here, I believe is both materialistic and ethical; as material corruption includes mischief on earth and around it, and ethical corruption means self and moral corruption.

To add to all these views that each civilization has its characteristics, its features, its morals, and its practices, Islam is unique in this, as Islam sees man as a whole, body and soul in full balance, none overweighs the other, as he did not worship the material, nor invented priesthood. Islam has taken care of man before he was born, when choosing a wife or a husband, at marriage, when he was a sperm drop, a baby, young, and old, Islam put to him a very accurate disciple system of life, taught him how to eat, drink, dress, treat himself, his Lord, his family, and his community. Islam has put to him goals in life - as it is a farm for the hereafter, to harvest from what his hands grew, and Islam was able to introduce a civilization to the world, with which Europe progressed from its dark ages with the help of the Islamic doctrines, but the Muslims slackened down and left Europe to lead the ship of scientific development. It may be that our interest in calling medicine by the Islamic Medicine, came as a symbol to awaken the Islamic world, and tell them that there is a lot in Islam in all fields; economic, architect, arts, cosmetic, medical... etc. and their commitment to Islam will bear fruits, too. One objective of choosing this name to medicine is the human deviations in practicing medicine in the West, but the East has to have a loud voice to awaken it and shake it; that is the voice of Islam, by providing the right opinion in these practices, especially when we lost the lead of materialistic science, but we can still provide it with

what purifies them and saves them from deviation, this is by means of the enlightened Islamic views. Moreover, the communication revolution has made the world a small village, knowing what happens all over it by the second... these developments are knocking our doors, thus we must be aware of it and give the Islamic view point in it, showing the advantage of Islam which differentiates between what is right from what is not.

The Lord knows what the inner-self whispers, as He is nearer to him than his vein, and He is the maker of his inner-self, and He directed him to his success, as He says'

"BY THE SOUL, AND THE PROPORTION AND ORDER GIVEN TO IT. AND ITS ENLIGHTENMENT AS TO ITS WRONG AND ITS RIGHT. TRULY HE SUCCEEDS THAT PURIFIES IT, AND HE FAILS THAT CORRUPTS IT".

(Al-Shams: 7).

The Almighty knows what the corrupt eye sees and what is hidden in the hearts.

Some people suggested that we call it THE ARABIC MEDICINE, in order not to distort the picture of Islam, as a result of misdemeanor of some practitioners, but this name might lead to the understanding of the use of medicinal plants and ancient medication practices, and this has its shortages, as well as its advantages, too, and because most of those who enriched the Islamic movement were not from the Arabic environment, like Al-Razy - from Al-Rey, Ibn-Sina - from Russia, and Al-Bukhary - from Tashkand... etc and thus we'll enter into the vertigo of apartheid, but Islam had engulfed them all. Moreover, if we want to discuss the point of view of Islam in modern things, on what ground shall we argue? Are there Arabic foundations? or, all the foundations taken from the Islamic Law (Shareeaa)? Thus the best name was "THE ISLAMIC MEDICINE", which is nearer to the fact, as for the fear of the misbehaviors, which might be alluded to Islam, wrongly, we know

that all Adam's sons are sinners, and the best sinners are the repentants, we are in a stage trying to erase eras of Islamic decay and weakness, we want to contribute to Islam and to be affiliated to it again, as well as to revive its name and face all over the world, and to prove that its doctrines are applicable, and their consequences are guarantee for man's well being and prosperity.

The Organization aims, also, at retrieving the Islamic behavior which was defined to Man by Islam, and make part and parcel of his daily conduct; if cleanliness, for example, is part of the belief, as said by the prophet (ﷺ), we find our Islamic states are the least countries enjoying and abiding by this Islamic ordinance, although it is the main road to health, and there are many wise sayings which organize the life of the Christians as well as the Muslims in order to lead a healthy and clean life, in the same way the orders and prescriptions in Islam are all related to man's psychological, social and body health; like prayer, fasting, Zakat, Haj, and others of the ordinances that have spiritual meanings which invests in Man tranquility and protects him form psychological and body diseases. There are many researches reinforcing these hypotheses, and the things that Islam forbids us from doing are essentially for our sake, we are not far away from what the world is suffering from narcotics, alcoholic drinks and AIDS which Islam prohibited.

We also wanted to utilize the plants which we have as a gift from the Lord, and Muslims have surpassed the world in this field, thus they kept their heritage of plants for the future generations, moreover they added and developed it. They wrote many books from which the Europeans took and translated and utilized till the 19th century; all their experiments and observations built on high scientific standards: Al-Hawy is considered the first scientific clinical encyclopedia in the history of the medical sciences.

Islamic civilization, at that time, was able to open its arms welcoming every active worker, Muslim or non-Muslim, as Islam

has no discrimination, and no coercion in religion, no one is better than the other except by worship and good deeds, thus scientists migrated to it from east and west to add to its sciences.

I'll mention here, only, the testimonies of some Western scientists for the Islamic civilization:- "Froje Garoody" talks with sadness and grief about western Civilization; he said. "The Western civilization is dying and committing suicide because it deviated from following the natural disposition; the instinct, and its masters considered man the director of the nature which he ruled, but after five centuries of the experience we found out that Nature is the main store of the primary materials and the place for man's leftovers, this made us always destroy nature, and this is against what the Holy Quran decided, as it decided that man is the Lord's heir on earth, and man is concerned with keeping natural balance"; then he says; "Our present western civilization is dying, not because it is short of means, but because it lacks goals". Man began to threaten himself with annihilation, and the result is the destructive weapons that man possesses are enough to destroy the planet earth one hundred times, what poor creatures we are!

This civilization is carrying in its womb the causes of its destruction, on the contrary of the Islamic civilization because the Islamic civilization is coming from the Lord who made it, not man, nor is the Islamic civilization an extension of history, but a revelation from the Lord to His prophet (ﷺ) through the Holy Quran, dictating a Holy Constitution satisfying the body and the spiritual needs of the human beings, then following this came the wise sayings of the prophet (ﷺ) to explain the quranic doctrine, thus everything became clear, the lawful is clear and the unlawful is clear, and the difference between them is clear. The world is about to face a crisis due to its losses from addictions, as the costs of these addictions reached 14 billion $ in one year in the USA only, and these losses were in work hours, accidents, family problems... etc.

due to the addiction of narcotics or alcoholic drinks, which Islam prohibited. This big sum of lost money is more than the revenue of many countries, and the world will face more than 40 million individuals inflicted with AIDS by the year 2000, and 10 million orphans; the WHO estimates the number will be doubled, nevertheless, virtue is absent, chastity killed, and they don't know where they are going... and no body knows!

Max Mayerhoof testifies: "The Islamic medicine has reflected the sun shine which was setting in Greece, and the moon glittered in the sky of the dark ages, and other stars brightened by themselves and lit the gloomy dark sky, then the moon went down and the light of the stars waned in the revival age, but their traces are still there, to be felt in the civilization of today.."

Montgomry Watt said; "I'm not going to look at Muslims as a barbaric army invading Europe, but I'll consider them the representatives of a civilization which achieved great successes all over the world, spread them to their neighbors. The Europeans are not appreciating their debt to the Islamic Civilization!! They even try to find faults with the volume of the Islamic effect and its importance in our cultural heritage, forgetting, again, that our good relations with the Arabs and the other Islamic nations calls upon us to be aware, to the end, that we owe them, not to mention this truth, or its denial is not right..."

Montgomry Watt didn't stop at that, but he added, "Our following the Arabic Medicine, which lasted till the 15th and the 16th centuries is evidently clear in the printed books, and the first of these books was explanations of the 9th chapter of the Principles of Al-Razy, then followed the printing of Ibn-Sina for three times, before Galinos, and till the year 1500 sixteen editions of "Al-Kanoon", the "Law". The statistics show that the quotations and extracts found in the early European writings are evidence that the

impact of the Arabic books surpassed and surmounted the Greek one.

He says, too, "Islam in essence is not only a mere religious movement, but it is also a human value embedded in life of the peoples who embraced Islam, or joined it, it was a kind of unique human existence in the world as the conditions of the Islamic openings were to permit the other people to continue practicing their former habits, laws, and languages, for paying taxes (Jiziah), these Islamic rules strengthened the relations between the Muslims and the peoples of the countries they conquered, thus the people continued to practice sciences, arts and especially medication.

These three testimonies are only a sample, there are a lot of others for which there is no space to quote here, but in time we will.

In addition to this, the last WHO statistics mention that 25-30% of the diseases from which man suffers nowadays are caused by the side effects of the chemical medicines, as well as their high prices, and the expertise which they need to manufacture. Contrarily, however, our Islamic countries enjoy a suitable weather for the medicinal plants to grow and treat a lot of diseases. All we need are issuing political decrees as China and India and other nations which produce these medications in the most modern fashion.

This is a short synopsis about the idea of Islamic Medicine, and to reinforce this idea, we invited a group of Muslim thinkers to take part in many conferences to write in this field, and we have received a lot of their contributions which will be published in due course of time, under different headings.

SUMMARY OF THE RESEARCHES IN THIS BOOK

This book consists of twelve exhaustive research papers dealing with the pharmacological, toxicological, biochemical, and clinical evaluation of different herbal formulations used in various diseases, namely - chronic bronchitis/asthma, rheumatoid arthritis, peptic ulcer, hypercholestrolaemia, ureteral colic, vitiligo, Bell's palsy and intestinal amoebiasis.

The first paper by Sabir and assoicates deals with the systematic investigation on the pharmacological basis of therapeutic action of a herbal formula in the treatment of chronic bronchitis and asthma. The herbal formual consists of nine plant ingredients, namely - dried fruits of *Vitis vinifera, Zizyphus vulgaris, Cordia latifolia, Malva sylvestris,* seeds of *Althaea officinalis,* rhizome of *Glycyrrhiza glabra,* whole plants of *Lavandula stoechas* and *Adiantum capillus veneris* and flowers of *Viola odarata.* The formulation has been found to exert antihistaminic and smooth muscle relaxant effects, togetherwith the augmentation of responses of catecholamines. Further, it inhibited the Schultz - Dale reaction, reduced the release of pharmacologically - active substances from the lungs induced by the immunological challenge or compound 48/80, appreciably stabilized the mast cells, inhibited the cutaneous hypersensitivity response and markedly suppressed the histamine-induced bronchospasm. These observations convincingly support the clinical usefulness of this herbal formulation in the treatment of chronic bronchitis and bronchial asthma.

The next five papers embody various studies conducted on a herbal formula used in rheurmatoid arthritis, containing dried roots of *Withania somnifera* and *Pyrethrum indicum,* corm of *Merendra persica* and rhizome of *Alpinia glanga.* El-Gindy and co-

workers have investigated its effect in different pharmacological models of inflammation and found that it produced highly pronounced suppressive effect against carrageenan-induced oedema of the rat hind paw and carrageenan-initiated release of histmine and 5-hydroxytryptamine. It also inhibited the cotton pellet induced granulation tissue formation and the adjuvant-induced arthritis. Its effect in these models was comparable to that of oxyphenbutazone. In a related study, Sadiq *et al* observed that this formula exerts stabilizing effect on lysosomal membrane system and also prevents the leakage of LDH from SRBC, and its effect was similar to that of acetyl salicylic acid.

Encouraged with the above-mentioned studies, Nazimuddin and associates investigated whether, like other nonosteroidal antiinflammatory drugs (NSAIDs), the herbal formulation used in rheumatoid arthritis exerts ulcerogenic effect. Notably, acute and chronic administration of the formula upto a period of 1 to 5 months did not exhibit any abnormality, erosion or ulceration either in the cardiac or pyloric portion of the stomach in rats of either sex. Incidentally, it protected the ulcer formation induced by pyloric ligation togetherwith significant reduction in total and free acid production. It also protected the stress - induced gastric lesions as also that induced by indomethacin, and its effect was comparable to that of cimetidine.

The convincingly promising anti-inflammatory and antiulcerogenic effects of the herbal formula for rheumatoid arthritis prompted Elmazar and colleagues to evaluate its toxicological effects, particularly the functional and behavioural teratological aspects and reporductive toxicity, in rats and mice. They did not observe any gross malformation or preimplantation embryonic loss nor any mutagenic effect. However, based on certain functional

alterations, the authors advised that the formula may be avoided during pregnancy and lactation period.

In the seventh paper, Qureshi *et al* have inevestigated the effect of minor components of plant materials, garlic and ginseng root on lipid and cholesteroal matabolism in chickens and concluded that these may have therapeutic potential for those individuals who for genetic or purely dietary reasons are inclined towards abnormally high blood cholesterol concentrations.

The eighth paper by Rahim and associates describes the effect of certain folk medicine agents (Zea Maize hair, Jerusalem stone, Halphabar, *Ammi visnaga, Ambrosia maritima,* Barely and *Petroselinum crispum*) on the urodynamics and ureteral activity in anaesthatized dogs. They observed that some of these folk medicines had an inhibitory effect while others had stimulatory action on ureteral activity; most of the agents tested produced a diuretic effect.

The next two papers deal with the clinical evaluation of certain herbal formulations in the treatment of vitiligo (*Bars*) in Kuwait (Al-Jerawi *et al*) and India (Iqbal Ali *et al*). Both the group of workers observed that these formulations were effective in the treatment of both localized and generalized vitiligo irrespective of age and sex of the patient and the duration of occurrence. In majority of cases, pigmentation appeared at the affected sites without any observable side effects.

Studies on the clinical evaluation of herbal formulations in the treatment of Bell's palsy (*Laquwa*) has been described in the eleventh paper by Siddiqui and Hasan. Efficacy of *Lavandula stoechas* and *Commiphora mukul* was investigated. It was found that these plants were highly effective as majority of the patients

were completely recovered within 21-80 days of therapeutic schedule.

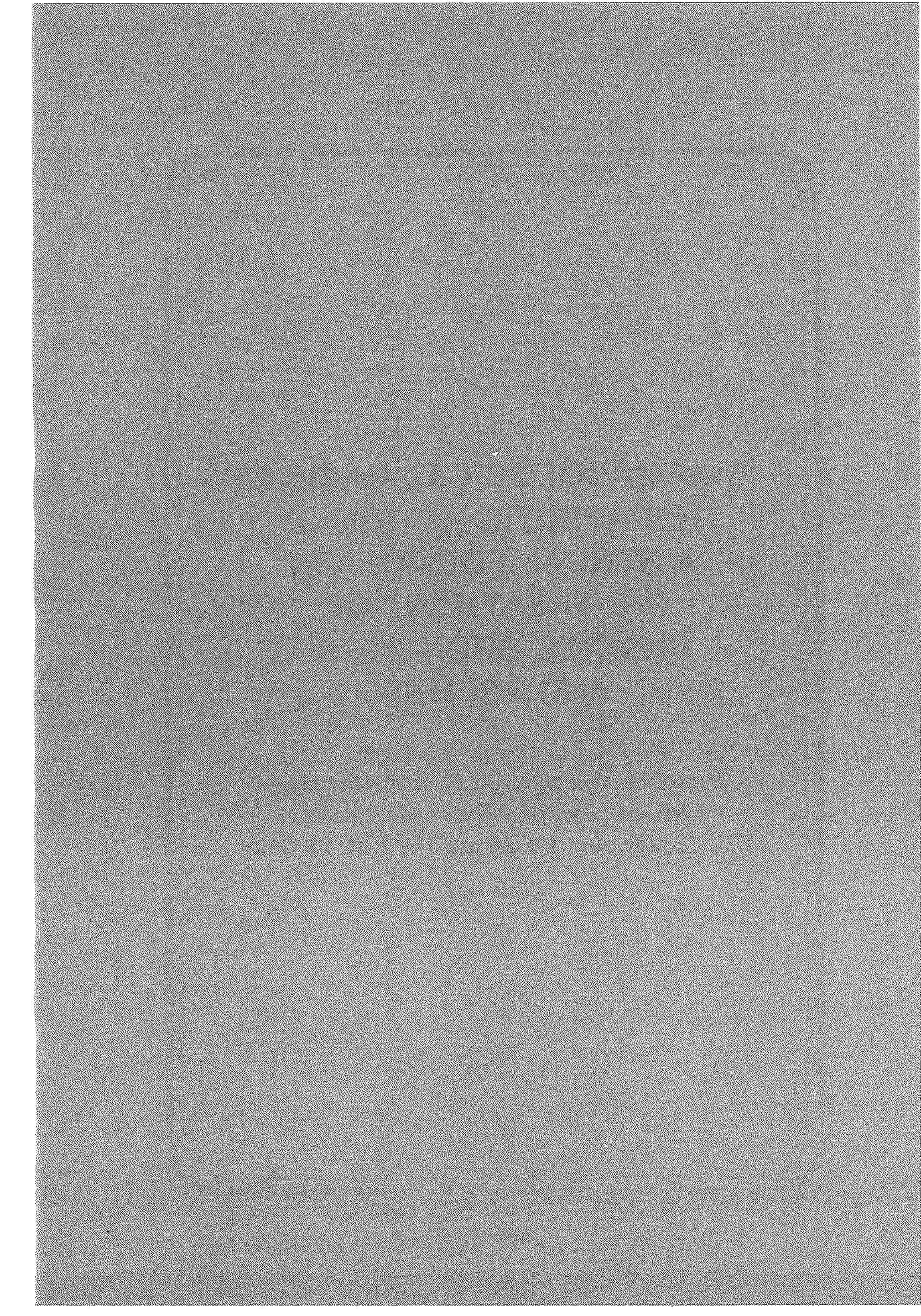
In the twelfth paper, Beg has described the clinical efficacy of a herbal medicine in the treatment of intestinal amoebiasis. He found that the patients suffering from *Entamoeba histolytica* and *Giardia lamblia* successfully responded to herbal therapy, and its effect was almost comparable to that of metronidazole. Unlike metronidazole, herbal drug did not prduced any side effects.



PHARMACOLOGICAL BASIS OF THERAPEUTIC ACTION OF A HERBAL FORMULA IN THE TREATMENT OF CHRONIC BRONCHITIS AND ASTHMA

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Abstract

The crude aqueous extract of the herbal formula antagonised the spasmogenic effect of histamine on the isolated guinea pig ileum. It also exhibited, at higher doses, a blanket activity against acetylcholine, carbachol, 5-hydroxytryptamine, bradykinin, prostaglandins E_1, E_2 and $F_1\alpha$ and potassium chloride. It inhibited the contracile effect of $CaCl_2$ on depolarized ileum. On the rabbit jejunum, the extract induced dose-dependent relaxation; it also sensitized the tissue to the relaxile effects of epinephrine and isoprenaline, and inhibited the contractile effects of acetylcholine, carbachol and histamine. On the rat fundus, it effectively antagonised the contractile effects of 5-hydroxytryptamine and prostaglandins $F_1\alpha$ and $F_2\alpha$; on the rat uterus, it inhibited the contractions induced by PGE_1. on the guinea pig atria, the extract produced a stimulant effect but in substimulant doses, it reversibly inhibited the positive inotropic and chronotropic effects of histamine and isoprenaline.

The extract inhibited the Schultz-Dale reaction on the isolated ileal pieces of ovalbumin-sensitized guinea pigs; the degree of inhibition of antigen-induced contraction was dependent upon the dose of the extract. It also prevented the release of histamine (pharmacologically-active substances) from the lungs of sensitized

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* Bulletin of Islamic Medicine, 5: (172-190), 1988.

guinea pigs challenged *in vitro* with specific antigen; it had similar effects on the release induced by compound 48/80. Further, it markedly reduced the degree of degranulation of messenteric mast cells induced by compound 48/80. To a separate group of guinea pigs, the extract was administered orally, daily, 4 days before and 3-4 weeks after sensitization. The ileum pieces from extract treated animals responded adequately to the antigen as also to histamine. However, the release of histamine from the lung tissues of these animals, challenged *in vitro* with specific antigen was significantly lower than the non-treated animals.

The extract markedly increased the latent period of histamine aerosol-induced bronchospasm and the resultant convulsions in guinea pigs and protected 40% animals from dying. It inhibited the cutaneous hypersensitivity response in guinea pigs as also the increase in cutaneous capillary permeability induced by histamine, bradykinin or 5-hydroxytryptamine, and, to some extent, facilitated the resorption of fluid accumulation at the site of histamine or antigen injection.

The extract was devoid of anti-inflammatory effect. It had no effect on the travel rate of charcoal meal in the gastrointestinal tract of mice but significantly inhibited the carbachol-induced increase in the gastrointestinal motility. It did not affect the magnesium sulphate-induced fluid formation in the rat intestinal loop.

It did not produce any adverse side effect upto a daily dose of 40 gm/kg fed for 3 days in rats and upto 20 gm/kg fed for 4 weeks in guinea pigs.

INTRODUCTION

In the traditional system of medicine, numerous plants are reputed, for over the centuries, to exert beneficial effects in diverse types of acute and chronic affections of the respiratory tract[1,2]. Of these plants, one formula composed of nine ingredients, namely-

dried fruits of *Vitis vinifera, Zizyphus vulgaris, Cordia latifolia, Malva sylvestris*, seeds of *Althaea officinalis*, rhizome of *Glycyrrhiza glabra*, whole plants of *Lavandula stoechas* and *Adiantum capillus veneris* and flowers of *Viola odorata*, has been recommended by the traditional medical practitioners for the treatment of chronic bronchitis and bronchial asthma[3,4,5,6]. Incidentally, many of these ingredients are also used, in one or the other combinations, in the treatment of chronic rhino-sinusitis, pharyngitis, laryngitis and pneumonia, and for other types of chronic catarrhal affections. Allama Kabeeruddin[7], the highly renowned philosopher-physician, classified these ingredients into 3 groups; this categorization was based on the works of his predecessors as well as on his own personal clinical experiences. Accordingly, he described that (i) *Viola odorata, Zizyphus vulgaris, Cordia latifolia, Malva sylvestris*, and *Althaea officinalis* act as a demulcent, relieve the irritation in the nose, throat and bronchi, suppress the bouts of cough and relieve the spasms of bronchial tube[7a], (ii) *Lavandula stoechas, Adiantum capillus veneris* and *Glycyrrhiza glabra* produce resolvent, deobstruent and expectorant effects, subside swelling of respiratory passage, dilate the bronchial tube and produce broncholytic effects[7b,c]; (iii) *Lavandula stoechas* and *Viola odorata* with *Vitis vinifera* act also as laxative and carminative and thus reduce the intra-abdominal pressure over the diaphragm and intrathoracic organs[7d] thereby relieving the chest distress by complimentary action. Notably, the decoction of this formulation is being used effectively to control the incidences of chronic bronchitis and asthmatic attacks in patients attending certain clinics of herbal treatment*. However, studies on possible mechanism of its action

* Goverment Nizamia General Hospital, Hyderabad (India) and in over 200 Government dispensaries in Andhra Pradesh (India): Also, at the Islamic Centre for Medical Sciences, Ministry of Public Health, Kuwait.

are apparently lacking. The present investigation was, therefore, undertaken to systematically evaluate the pharmacological basis of therapeutic action of this formula in chronic bronchitis and bronchial asthma or the chronic airway obstruction.

MATERIALS AND METHODS

Extraction procedure:

The fruits of *Zizyphus vulgaris* and *Vitis vinifera* were deseeded, the remaining plant materials crudely pulverised, and extracted with distilled water at room temperature (22-24°C) for 24 hours; to each 100 gm of material 1 litre water was added. Thereafter, the supernatant was filtered through muslin cloth. The filtrate was evaporated, under the running exhaust, to desired concentration and kept frozen for subsequent use, when it was pre-warmed to room temperature.

Animals:

Inbred strains of rats, mice, guinea pigs and rabbits of either sex were used in the present study. The animals were maintained on standard pellet diets and *ad lib* water. Guinea pigs were also given additional multivitamins mixed with drinking water.

Isolated tissues:

Pieces of guinea pig ileum, rabbit jejunum, oestrogenized rat uterus[8], rat fundus[9] depolarized guinea pig ileum[10], and isolated guinea pig atria[11], were suspended in thermostatically controlled 10 ml capacity isolated tissue bath containing Tyrode (37°C), Ringer (37°C), deJalon (31°C), potassium Ringer (37°C) and Ringer-Locke (30°C) solutions respectively; the solutions were continuously bubbled with air. The contractions were recorded on a smoked drum with a simple lever (magnification 1:7). For oestrogeniza-

tion[12], adult female non-pregnant rats received oestradiol benzoate (0.1 mg) in 1 ml arachis oil intraperitoneally, daily on 2 successive days; the animals were sacrificed 24 hr after the last injection. For guinea pig ileum and rabbit jejunum, the animals were denied food, but allowed *ad lib* water, for 24 hr before sacrifice.

A 3-5 minute time cycle was followed. Contact period for different spasmogens was 25-30 seconds on guinea pig ileum and rabbit jejunum, and 60-90 seconds on rat uterus and rat fundus. The extract was allowed to act on the tissue for 60 seconds before addition of spasmogens. The tissue was repeatedly washed with fresh physiological solutions after each addition of drugs until the original state of the tissue was obtained.

Anti-Anaphylactic studies (Schultz-Dale reaction):

(a) *Sensitization procedure*: Guinea pigs weighing between 500 and 600 gm were used. The animals were actively sensitized[13] using fresh egg-white (3-5 ml of 25% v/v solution in normal saline) intraperitoneally. Booster dose of antigen was administered via the same route 3 days after the first administration.

(b) *Treatment schedule*: The sensitized guinea pigs were divided into control group and a test group. The former were kept on water while animals in the latter group were each given, in place of water, 50 ml of diluted extract (corresponding to 20 gm of plant material extract) per day. The treatment schedule was followed throughout the sensitized period of 3-4 weeks. Another group of non-sensitized animals were fed with same quantity of the extract for similar periods.

(c) *Anaphylactic contractions*: The animals were sacrificed after the sensitization period. The entire ileum was dissected out, cut into 3 cm long pieces and cleaned. The pieces were mounted vertically in a 10 ml organ bath as per the procedure described above for the isolated guinea pig ileum.

Anaphylactic contractions were elicited by the addition of varying doses of antigen and recorded. The antigen (egg-white 25%) dose ranged between .01 to .04 ml/ml bath fluid. Individual piece was discarded after one exposure to the antigen. The extract was added 1 minute prior to the antigen.

Mediator release from the lung tissue:

Control and sensitized animals were sacrificed by cervical dislocation followed by ex-sanguination to prevent blood from being aspirated into the lung[14]. The dissected lung was washed free of blood by passing aerated Tyrode solution through the pulmonary artery.

Approximately 0.5 to 1 gm wet weights of the washed lung tissues, kept in warm aerated Tyrode solution (100 mg tissue per 2 ml Tyrode), were challenged with 0.2 ml of antigen. Simultaneously, equal number of pieces were exposed to 200 mg of the extract for about 30 minutes; thereafter, the tissue was transferred to normal Tyrode solution and challenged with 0.2 ml of antigen.

In another set of experiments, the lung pieces from nonsensitized guinea pigs were exposed to 0.1 ml of 0.1% compound 48/80; concomitantly, equal number of pieces were exposed first to 200 mg of the extract and then to compound 48/80 (0.1 ml of 0.1% solution).

The exposure time either to the antigen or to compound 48/80 ranged between 15 to 20 minutes after which 1 ml aliquots of the solution were withdrawn and assayed on the nonsensitized atropinized guinea pig ileum. A dose-response curve using varying concentrations of histamine dihydrochloride, up to 30 ng/ml, on the same piece of ileum was plotted to obtain a calibration curve. The quantity of mediator released from the lung was estimated as histamine equivalents from this curve. For the release of mepyramine resistant spasmogens, the aliquots were assayed in the presence of mepyramine maleate at a final bath concentration of 10

ug/litre. Contractions caused by mepyramine-resistant spasmogens released per gm of lung tissue were expressed as per cent the contractions caused by 30 ng/ml of histamine dihydrochloride in the same non-sensitized ileal piece.

Cutaneous hypersensitivity:

The guinea pigs were sensitized with egg albumin as per the method described above. Three weeks after sensitization, Evans Blue (5 mg/kg) was injected, intravenously, either through the marginal ear vein or penal vein, 30 minutes before the intradermal injections (in the prepared abdominal region) of the antigen. At some spots, varying concentrations of extract was injected 10 minutes prior to the injection of antigen at the same spots. The volume of fluid injected at any single site and at any single time was 0.1 ml. The increase in permeability (wheal formation) was assessed[15] at 15 min, 1/2, 1, 2, 3 and 4 hour post-injection. The intensity of the increase in permeability was graded as +, + +, + + + and + + + + indicating mild, moderate, intense and severe reactions[16].

Cutaneous capillary permeability:

The method described above was followed except that the guinea pigs were not sensitized and the antigen was not injected intradermally. This study was also done in rabbits[17]. Histamine, bradykinin and 5-hydroxytryptamine were used as standard capillary permeability-increasing agents. The effect of extract was compared with mepyramine maleate.

Messenteric mast cells:

The messenteric tissues from the normal (nonsensitized) guinea pigs were exposed *in vitro* to compound 48/80 for 30 minutes. In some experiments, the messenteric tissues were first exposed to the

extract for 30 minutes and then to compound 48/80 for another 30 minutes period. Thereafter, the tissues were spread over glass slides and stained[18]. The degree of degranulation was observed microscopically (x 40).

Histamine-induced bronchospasm:

Guinea pigs of either sex (600-700 gm) were exposed[19] to finely atomized mist of histamine dihydrochloride (1.5 per cent w/v) into aerosol chamber (Ugo basile, Italy). Compressed air at a constant pressure of 200 mmHg was used to operate the nebuliser. The extract (20 gm/kg) was fed orally, daily, for 3 days, before the animals were exposed to the histamine aerosol. In other group of animals, mepyramine maleate (5 or 15 mg/kg) was injected intraperitoneally 30 minutes before exposure[20]. The time of onset of anoxic convulsions was recorded. The animals not showing convulsions upto 6 minutes after the start of histamine aerosol were regarded as completely protected.

Carrageenan-induced inflammation:

Acute inflammation was induced in the right hind paw of rats (either sex; 200-300 gm) by single sub-plantar injection of 0.1 ml of 1% solution of carrageenan[21]. The paw volumes were determined by means of a plethysmometer (Ugo basile, Italy). Animals were pretreated orally, daily, with different doses of the extract for varying periods, the last administration being 1 hr before carrageenan.

Cotton pellet granuloma:

Sterile cotton pellets 10 mg each were implanted[22] bilaterally in pectoral and groin regions of rats under ether anaesthesia. The pellets were dissected out on the 7th day and wet and constant dry

weights recorded. Drugs were administered once daily, orally, starting one day prior to the implantation of pellets.

Travel of charcoal meal:

Adult mice (25-30 gm) of either sex were denied food for 24 hr but offered water *ad libitum*. Charcoal meal[23] was prepared by suspending 1 gm finely powdered activated charcoal in 10 ml of 25% gum acacia in water. The mice were given the extract, orally, 30 minutes before the charcoal meal (0.2 ml). Twenty minutes after charcoal meal feeding, the mice were sacrificed[24]. The abdominal cavity was opened and the entire small intestine from pylorus to ileocaecal junction was then gently freed by cutting the intestinal edge of the messentry. The freed intestine was gently placed, without stretching, in a straight line on a white filter paper. Length of the entire small intestine as also the portion traversed by dark-coloured charcoal meal were measured. Percentage of the small intestine length travelled by charcoal was then calculated.

Fluid accumulation in intestinal loop:

Adult rats (200-300 gm) of either sex were denied food for 24 hours but allowed *ad lib* water. Abdomen was opened under pentobarbitone anaesthesia and, extending from 3 cm below the pylorus, a 30 cm long intestinal loop was prepared by two ligatures[25,26]. Magnesium sulphate (2 ml of 15% solution) was injected in the loop. In one group of animals, the extract was injected directly into the loop 10 minutes before magnesium sulphate; in another group of animals, the extract was fed one hour before ligation and magnesium sulphate injection. Loop fluid was collected after 1, 2 or 3 hr and measured.

RESULTS

Isolated tissues:

Guinea pig ileum:-

The extract did not exert any direct effect of its own on the isolated guinea pig ileum upto a dose of 100 mg/ml. It, however, inhibited the contractions induced by histamine (23 experiments). The antagonism was proportional to the dose of the extract. Thus 10, 20, 40, 60 and 80 mg/ml of the extract inhibited the histamine response (10 ng/ml) by 12.32, 22.23, 57.94, 70.05 and 86.93 per cent respectively. The ED_{50} for histamine was calculated to be 35.8 mg/ml. Antagonism by the smaller doses of extract was quickly reversible but by the higher doses it persisted for longer time. Antagonism could be repeatedly elicited on the individual tissues and it could be overcome by increasing the dose of histamine.

At relatively higher doses, the extract also produced dose-dependent inhibition of the spasmogenic effects of acetylcholine (6 experiments), carbachol (7 experiments), 5-hydroxytryptamine (5 experiments), bradykinin (3 experiments), prostaglandins E_1 (4 experiments), E_2 (5 experiments), and $F_1\alpha$ (2 experiments) and potassium chloride (4 experiments). The ED_{50} for respective agonists were calculated to be 60.2, 65.4, 50.2, 75.3, 68.2, 70.2, 64.3 and 61.6 mg/ml respectively.

On the depolarized tissue (5 experiments), the extract (10-30 mg/ml) reversed the carbachol-induced contracture and inhibited, by about 30-60%, the calcium chloride (1 mg/ml) induced contractions (4 experiments).

Rabbit jejunum:-

In doses ranging between 10 and 30 mg/ml, the extract inhibited the pendular movements and produced dose-dependent relaxation of the tissue (6 experiments). No tachyphylaxis was observed on

repeated exposure of the tissue to the same dose of the extract. In two experiments, the extract produced a triphasic response, that is, slight initial relaxation followed by contraction and then profound relaxation. The relaxile effect was not altered by propranolol (0.5 ug/ml; 2 experiments) or phenoxybenzamine (1 ug/ml; 2 experiments) nor by their mixture (3 experiments); also it was not blocked by pentolinium (5 ug/ml; 1 experiment). However, the extract sensitized the tissue to the relaxile effects of adrenaline (0.5 ug/ml; 6 experiments) and isoprenaline (0.3 ug/ml; 2 experiments), in that, it augmented the responses of adrenaline and isoprenaline by about 30 and 40 per cent respectively. But, on the other hand, it antagonized the contractile effects of acetylcholine (1 ug/ml; 3 experiments), carbachol (0.5 ug/ml; 4 experiments) and histamine (7 ug/ml; 4 experiments).

Rat fundus:-

In 2 out of 7 experiments, the extract (10-30 mg/ml) produced slight contraction of its own. However, in the subcontractile doses (1, 3 and 10 mg/ml) on these tissues as also on the remaining 5 tissues, it inhibited the 5-hydroxytryptamine induced contractions by 23.0, 54.2 and 80.3 per cent respectively. At 5 and 10 mg/ml doses, it inhibited the contractions induced by $PGF_1\ \alpha$ or $F_2\ \alpha$ by 30.7 and 70.2 per cent respectively. The extract induced inhibition was reversible and could be overcome by increasing the dose of the agonists.

Oestrogenized rat uterus:-

The extract did not exert any direct action of its own on the oestrogenized rat uterus upto a dose of 50 mg/ml (3 experiments). It, however, inhibited the contractions induced by PGE_1 (0.6 ug/ml; 3 experiments) by 20.6, 37.2 and 60.4 per cent at 3, 6 and 10 mg/ml doses respectively. Thus, the antagonism was roughly proportional to the dose of the extract and was quickly reversible.

Guinea pig atria:-

The extract had no effect of its own on this preparation upto a concentration of 1.0 mg/ml of the bath fluid. However, at 5 mg/ml concentration, it produced a positive inotropic and chronotropic effect. At a concentration devoid of any effects on the spontaneous activity (1 mg/ml), the extract reversibly inhibited the positive chronotropic and inotropic effects of histamine (3 ug/ml) by about 80 per cent and that of isoprenaline (0.1 ug/ml) by about 80 per cent.

Anti-Anaphylactic effect:

Three weeks after active sensitization, ileal pieces of the sensitized guinea pigs (6 experiments) responded by contraction when challenged with the antigen (egg-white 25% v/v; 0.2 ml). Pieces of the intestine kept in Tyrode solution at 6-8°C for upto 24 hr did not lose sensitivity to the antigen.

The height of contraction induced by the antigen was dose-dependent. The extract markedly reduced the antigen-induced contraction at a dose of 3 to 10 mg/ml. At higher concentrations (30-100 mg/ml), the extract completely abolished the antigen response (5 experiments). Subsequent addition of antigen, after the extract was washed out, did not elicit any contraction though it remained sensitive to histamine. However, the ileal pieces from the extract-pretreated animals (4 experiments) were as sensitive to the contractile effect of antigen or histamine as were those from the non-treated (control) sensitized animals.

Mediator release from lung tissues:

The quantitative estimation of the amount of spasmogens released anaphylactically or chemically was done by assay on the isolated ileal pieces from nonsensitized guinea pigs. The results are presented in Table I. The amount of spasmogens released

chemically under the influence of compound 48/80 (8 observations), *in vitro*, was manifold greater (3.58 ± .14 ug/gm) than the controls (0.43 ± .04 ug/gm; $P < .001$; 10 observations); however, the extract almost completely inhibited (0.53 ± .13 ug/gm) the compound 48/80 - initiated release (8 observations). Curiously, the amount of spasmogens released from the lungs of egg albumin-sensitized animals was significantly ($P < .001$) higher (1.36 ± .11 ug/gm; 8 observations) than the nonsensitized animals (0.43 ± .04 ug/gm).

Exposure of the lung tissues, from the sensitized animals, *in vitro*, to the antigen, resulted in significant ($P < .001$) increase in the spasmogen release (2.78 ± .13 ug/gm; 7 observations); however, the extract significantly ($P < .001$) reduced the antigen-initiated release (1.86 ± .09 ug/gm; 8 observations). Lung tissues from sensitized animals, fed on the extract during the course of sensitization, when challenged *in vitro*, with antigen also released lower amounts (2.14 ± .11 ug/gm; 5 observations) than those from the nontreated sensitized animals (2.78 ± .13 ug/gm).

In 5 experiments, the contractile effect of spasmogens was studied in the presence of mepyramine. Significant reductions ($P < .05$) in the degree of contractions were observed in mepyraminized tissues. However, still the spasmogens remained markedly effective even in the presence of mepyramine.

Cutaneous hypersensitivity:

In the sensitized animals, intradermal injections of the egg-white, in concentrations ranging from 5 to 25%, produced effects at the site of injection starting from initial blue discoloration to clear-cut wheal formation (5 experiments). The effect was discernible (±) within 15 minutes; by 30 minutes the intensity of reaction became moderate (+ +) reaching to highly severe (+ + + +) stage by the second hour after injection (Table II). Thereafter, the severity of reaction gradually declined to milder degree by the 4th hour.

However, effect of the antigen was not dose-dependent in that 5, 10, 15, 20 and 25% concentrations of the antigen produced similar degree of response. Local infiltration of the extract strikingly antagonized the antigen response during the entire 4 hour observation period (5 experiments). In the sensitized animals fed with the extract, the antigen produced only mild reaction which was significantly less than that produced in the nontreated animals (3 experiments). The antigen had no effect in the non-sensitized normal animals (2 experiments).

Cutaneous capillary permeability:

In the dose range of 1-10 ug, histamine dihydrochloride induced marked increase in the permeability (6 experiments). The effect of histamine was roughly proportional to the dose. The extract alone, upto a dose of 100 mg, did not produce any marked increase in the capillary permeability (4 experiments). However, it significantly reduced the histamine-induced increase in the permeability (Table III) and also facilitated the restoration of normalcy (6 experiments). For instance, whereas the effect of 10μg histamine alone persisted until 4 hours of observation period, its effect in the presence of extract was almost negligible at the end of that period. The inhibitory effect of 30 mg extract was comparable to 5-10 μg of mepyramine maleate; at 15 μg, mepyramine completely nullified the histamine effect (4 experiments).

Like histamine, bradykinin and 5-hydroxytryptamine also produced marked increases in the permeability which remained unaltered in the presence of mepyramine (Table IV). However, the extract did reduce the effects of both bradykinin and 5-hydroxytryptamine, though the reduction was comparatively lesser than that for histamine (3 experiments).

Oral administration of the extract, 20 gm/kg, daily for one week, also appreciably inhibited the responses of intradermally

injected histamine, bradykinin and 5-hydroxytryptamine (2 experiments).

No sign of tissue damage or necrosis was observed at the site of extract injection.

Histamine-induced bronchospasm:

The results are presented in Table V. All the 5 animals in the control group developed breathing difficulty and anoxic conclusions after mean exposure time of 68 seconds to histamine aerosol and then died. At a dose of 20 gm/kg, the extract protected 40 per cent (2 out of 5) animals from developing respiratory distress or convulsions and death; the remaining three animals did develop the convulsions but the latent peroid of the onset of respiratory distress was significantly longer (128 seconds) than that in the controls (Table V).

At a dose of 5 mg/kg, mepyramine failed to protect the animals against histamine-induced bronchospasm; all the 5 animals developed convulsions after mean exposure time of 64 seconds to histamine aerosol and died. At 15 mg/kg dose, however, mepyramine protected all the 5 animals from developing respiratory distress or convulsions and death (Table V).

Messenteric mast cells:

At a concentration of 10 μg/ml, compound 48/80 produced significant ($P < 0.02$) degree of degranulation ($38.0 \pm 9.84\%$) of messenteric mast cells as compared to those treated with normal saline alone ($4.0 \pm 2.1\%$). The extract completely protected the mast cells from getting degranulated by the compound 48/80 since in the presence of the extract, the compound could induce degranulation only of $4.8 \pm 2.78\%$ cells; this degree of degranulation was almost comparable to that of normal saline (Table VI). Four experiments were conducted in each set.

Carrageenan-induced inflammation:

Intraplanter injection of carrageenan caused significant increase in the paw volume due to oedematous swelling (10 animals). The increases in the paw volume at 1/2, 1, 2, 3 and 4 hr after carrageenan injection were 24.9, 27.7, 45.2, 73.4 and 67.8% respectively. Oral administration of the extract did not reduce the carrageenan effect. Two doses (20 and 40 gm/kg) of the extract were tested. In the animals receiving 20 gm/kg (8 animals) extract, the per cent increases in the paw volume at respective intervals were found to be 22.0, 26.4, 40.7, 72.6 and 68.4, and in those receiving 40 gm/kg (7 animals) these figures were 30.0, 34.8, 55.1, 68.4 and 81.2. However, brufen did significantly ($P < .001$) inhibit the carrageenan-induced oedema (8 animals), in that, in this group of animals the per cent increases were 9.5, 10.9, 19.7, 9.3 and 9.8.

Cotton pellet-induced granulation tissue formation:

In the control group of animals, the wet and the constant dry weights of the granulation tissue were 137.4 ± 4.21 and 35.3 ± 2.61 mg respectively (10 animals). Oral administration of the extract (20 gm/kg) throughout the one week experimentation period did not reduce the granulation tissue formation (10 animals); the wet and dry weight of the tissue in the extract-treated animals were 133.6 ± 3.84 and 34.7 ± 2.43 mg respectively. However, dexamethasone (8 animals) and brufen (7 animals) significantly ($P < 0.001$) reduced the granulation tissue formation; the weights in the respective groups were 56.2 ± 1.63 and 16.8 ± .82, and 74.3 ± 2.03 and 22.5 ± 1.36 mg.

Travel of charcoal meal:

In control group of mice, 64.0 ± 5.68 per cent length of the small intestine was travelled by charcoal (12 animals). The extract (20 gm/kg) had no marked effect on the motility since in the mice (10

animals) receiving extract the distance travelled by charcoal meal (58.5 ± 5.20 per cent) was almost similar to that observed in the controls. However, carbachol markedly increased the per cent distance covered by charcoal (8 animals) which was 74.37 ± 1.99, and this increase was completely, and significantly ($P < .001$), inhibited by the extract (10 animals). The results are presented in Table VII.

Fluid formation in the rat intestinal loop:

Magnesium sulphate solution (2 ml; 15% w/v) produced 5.44 ± 0.17, 5.23 ± 0.46 and 5.38 ± 0.23 ml fluid accumulation at 1, 2 and 3 hours respectively in the ligated intestinal loop of adult rats (5 animals at each interval). The extract (1 gm/loop) did not influence the course or volume of fluid formation. The volumes of fluid recovered from the loops pretreated with extract were 5.29 ± 0.34, 5.58 ± 0.49 and 5.16 ± 0.27 at the respective intervals (6 animals in each group). Almost similar volumes were recovered from the loops of those animals which were fed the extract (10 gm/kg) one hour before ligation of the intestinal loop and injection of magnesium sulphate (6 experiments).

DISCUSSION

The results of the present investigation on the herbal formulation indicate that the crude aqueous extract of the formula exerts antihistaminic effect. However, term "antihistaminic" is used for convenience and because, as in the previous reports reviewed[27], antagonism to histamine was the first to be detected and studied. The term does not imply the highly specific antihistamine action which characterizes synthetic or classical antihistamines. Nevertheless, in almost all the parameters covered in the present study its effect do suggest of certain degree of specificity against histamine but many more parameters need to be studied in greater details. On

the isolated tissue preparations, larger doses of the extract were required for antagonising the effects of acetycholine, carbachol, 5-hydroxytryptamine, bradykinin, prostaglandins and potassium chloride. This type of wide and varied antagonism is not uncommon to synthetic antihistamines[28] as also to naturally-occurring antihistamines[29,30,31]. The ability of naturally-occurring antihistamines to inhibit the smooth muscle contractions induced by so many agonists and antigen has been described as "the blanket activity"[27]. On the isolated guinea pig ileum and in certain other parameters covered in the present study, histamine manifests its action by acting upon H_1 receptors. The only preparation having H_2 receptors used in the present investigation was isolated guinea pig atria[32]. On this preparation, the extract inhibited the effect of both histamine and isoprenaline indicating a nonspecific effect on H_2 receptors. Nonetheless, it would be advisable, before arriving at such a conclusion, to conduct additional experiments envolving H_2 receptors particularly the Shay rat preparation. Synthetic antihistamines do not block the action of histamine on the gastric acid secreting glands; indeed, this anamalous stituation has led to the concept that histamine receptors on these glands are different from those on the intestinal smooth muscle[33]. It may be interesting to note that naturally-occurring anti-histamines from human and horse urine[34] and from frog skin[31] inhibit histamine-induced gastric acid secretion in mammals. Need for such a study is emphasized here particularly because of the fact that the decoction of the formula is recommended to be drunk preferably in the empty stomach.

Catecholamines, like adrenaline and isoprenaline, do inhibit the spasmogenic action of histamine and other agonists on the isolated smooth muscle preparations. On the rabbit jejunum, like adrenaline and isoprenaline, the extract produced a relaxile effect; however, this effect was not blocked by propranolol or phenox-

ybenzamine nor by their combination indicating thereby that the relaxile effect was not mediated through the activation of adrenergic receptors. Also, the extract-induced relaxation did not exhibit any degree of tachyphylaxis. These findings therefore suggest that the relaxant effect of the extract is probably due to its direct action on the relaxile elements of the smooth muscle independent of the involvement of the adrenergic receptors or catecholamine stores. These findings, render it unlikely that the antihistaminic principle in the extract is ephedrine or ephedrine-like. Moreover, the extract itself antagonised, at certain dose level, the effect of isoprenaline, as it did of histamine, on the isolated guinea pig atria.

However, the smooth muscle relaxant activity of the extract as observed on the rabbit jejunum is an interesting finding. Antagonism of certain agonists on the rabbit jejunum as also on depolarized ileum further strengthens the view that it acts directly on the relaxile elements.

The non-specific spasmolytic agents such as papaverine and polysorbates inhibit isolated guinea pig ileum contractions induced by diverse agonists including potassium chloride[35] which induces contraction by surface depolarization. In this respect, they completely resemble the extract. Therefore, it is reasonable to propose that in a normal isolated guinea pig ileum, the extract inhibits certain agonists by some mechanism at the cell membrane of the smooth muscles. On the other hand, in the isolated guinea pig ileum depolarized by prolonged exposure to excess potassium sulphate, the cell membrane is depolarized and non-functional and agents like calcium chloride which induce contraction in this preparation do so by directly acting on the contractile elements[36,37] inside the cell by initiating the excitation-contraction-coupling mechanism[38]. This calcium chloride-induced contraction is blocked by papaverine[35] as also by the extract. This finding suggests that, at the intracellular sites of excitation-contraction-coupling, the extract

reduces the availability of calcium. All these findings may also explain the clinically conspicuous antispasmodic action of the extract.

Like isoprenaline, the extract produced a positive inotropic and chronotropic effect on the isolated atrial preparations of guinea pigs. The nature of cardiac stimulant action of the extract is not clear since in substimulent doses it inhibited the stimulatory effects of both isoprenaline and histamine. This action needs further study.

Active anaphylaxis in the guinea pig was used in this study to find out the scientific basis for the clinically observed anti-asthmatic effect of the formula. There is a whole series of events in the anaphylactic reaction, starting with the production of reaginic (type of) antibodies and cluminating in the actual smooth muscle contraction[39]. A Schultz-Dale reaction is the contractile response of the sensitized smooth muscle preparation, *in vitro,* when challenged with antigen. In the present study, the extract effectively antagonized the antigen-induced contraction (Schultz-Dale reaction) of the ileal pieces of sensitized guinea pig. However, systemic (oral administration) of the extract through the course of sensitization period was not effective in that the ileal pieces from extract-fed, sentitized animals reacted adequately to the antigen. This finding, therefore, suggests that, in the doses used, the extract does not affect the generation/production of specific antibodies or their concentration in the target organs but interferes with the interaction of these antibodies with the antigen and thereby prevents the acute anaphylactic shock. The tissues once exposed to the extract and then challenged to the antigen remained unresponsive to subsequent addition of the antigen even after the extract was washed out; this finding suggests that neutralization of the antibodies was complete and total.

The lung is the target organ in asthma. Therefore, the release of mediators of anaphylaxis from this organ was studied. The

reduction in the amount of spasmogens released from the lungs, as observed in the present study, shows the effect of the plant materials on the target organ. The degree of contractions recorded, as presented in Table I, are most likely to be due to histamine release because of the shorter time course of the contractions which indicates that histamine is the major spasmogen released. The reduction in the total content of spasmogen as a result of the *in vitro* treatment with the extract is of significance, and so is the reduction in the amount of the amine released anaphylactically. With respect to the effects of histamine the basic difference between normal and hypersensitive individuals is the quantity of the released mediators which will elicit a pathological response. The same quantity will be innocuous in normal individuals and pathological for hypersensitive individuals. A mechanism for the reduction in the amount of mediators released either by decreasing the total content or by interfering with the release mechanism is of special significance as this would tend to correct the underlying derangement in the hypersensitive individual by decreasing the amount of mediators released to a level below the pathological level. The extract appears to affect both mechanisms of reduction, so that its effect is not only at the step of smooth muscle contraction but also at a step preceding the release of mediators.

The contractions recorded as a result of mediators released from the lung tissue in the presence of mepyramine show that the extract has effect also on the release of spasmogens other than histamine. The significance of this result lies in the fact that in man the release of spasmogens other than histamine is important in asthmatic attacks[40]. Histamine is known to be formed during the sensitization period and merely released on challenge[41], whereas the other mediators are known to be made *de novo* in response to the challenge[42]. The effects of the extract on the levels or effects of these mediators of anaphylaxis also indicate its active inferference in the

response to challenge. It would seem that the extract affects several steps in the sequence of events in anaphylaxis, and this could account for its prophylactic use in asthmatics.

Further, the extract also reduced the level of histamine release from the lungs exposed *in vitro* to compound 48/80. This compound acts like antigen at the cell surface, as suggested by their affinity for acid mucopolysaccharides present in the membrane[43], or the essential component of their action may be a mobilization of cellular calcium[44]. Our experiments on the isolated depolarized tissues have indicated that the extract might be interfering with the calcium-interaction with the cell membrane. It is likely that the effect of extract observed on the compound 48/80-induced release might as well be due to its action on the mobilization of cellular calcium.

In the present study, the extract inhibited the effect of compound 48/80 on the mast cells. Compound 48/80 is a potent degranulator of mast cells which contain besides histamine many other pharmacologically-active substances. These cells supposedly play a pivotal role in the causation of asthma or bronchitis because of their ability to produce and release mediators of broncho-constriction[45]. Therefore, the mast cell stabilizers have been considered effective in the prophylaxis of asthma. One of the outstanding examples is disodium cromoglycate, which is a unique drug that has no bronchodilator properties; it rather prevents bronchospasm probably by stabilizing mast cells so that release of preformed mediators by antigen-antibody reaction or other stimuli is inhibited[46]. An inhibitory action on antigen-induced production of histamine and SRS-A has been demonstrated in peritoneal mast cells *in vivo* and *in vitro*[47]. However, the action of suppressing histamine release is not a general one; histamine release from mast cells in response to releasing drugs such as compound 48/80 or bee venom is unaffected[48]. And, in this respect, the extract of the

formula defers with sodium cromoglycate since the extract prevented the release induced by compound 48/80 and also exhibited smooth muscle relaxant and bronchodilator properties. It would be interesting to study the effect of extract on antigen-induced degranulation of mast cells because even though histamine has been repeatedly identified as a prominent constituent of mast cells and is released in large quantities following antigen-antibody interaction[49], histamine is thought to be of relatively little importance due to the presence of other mediators[50]. Apparently, mast cells stabilizing effect of the extract does not appear to be nonspecific since it failed to produce stabilization of fresh red blood cells[51].

In the present study, a simple model for studying the cutaneous hypersensitivity response has been followed. This does not involve the sensitization procedures in which the antigen was presented either in an unusual physical state (e.g. absorbed on aluminium hydroxide gel) or in conjunction with certain adjuvant toxins (e.g. *B. pertussis*) nor when the animal body was subjected to particular stresses so that the corticosteroid levels were temporarily degraned[52]. In our experiments, the guinea pigs were simply sensitized by the intraperitoneal injection of egg-white and were used for test 3 weeks after sensitization. In the sensitized animals, the intradermal infiltration of the antigen produced effect which appeared to be specific because in the non-sensitized (normal) guinea pigs, the antigen failed to induce wheal formation. The extract, when injected locally prior to the injection of antigen at the same site, completely antagonized the wheal formation by the antigen. However, oral administration of the extract was relatively less effective in antagonizing the effect of antigen. This could be due to lower concentration of the extract reaching the cutaneous tissues as compared to its local injection.

The extract also inhibited the increase in cutaneous capillary permeability induced by histamine, bradykinin and 5-hydroxytryptamine. However, its effect against histamine-induced increase in permeability was more marked than that induced by bradykinin and 5-hydroxytryptamine suggesting greater affinity for histamine receptors than those for the remaining agonists. In the dose which blocked the histamine response, mepyramine had no inhibitory action against bradykinin and 5-hydroxytryptamine. Thus, in this respect, the extract exerted a wider range of activity against these agonists than mepyramine maleate. The antagonism of the increase in cutaneous capillary permeability as also of cutaneous hypersensitivity does not appear to be the nonspecific effect of extract on membrane permeability since in the rat intestinal loop experiment, it failed to affect the magnesium sulphate induced fluid accumulation which results from the altered permeability of intestinal mucosa due to osmotic tension.

The extract increased the latent period of histamine aerosol-induced anoxic convulsions and protected certain per cent of animals from death. Bronchial muscle of guinea pigs is exquisitely sensitive to histamine and bronchoconstriction leads to death. In clinical situation, altered reactivity of bronchial musculature triggers acute bronchospasm. Therefore, any drug or agent which can inhibit the histamine induced bronchospasm could be of promising value in the treatment of acute asthmatic attacks. The constrictor action of histamine on respiratory smooth muscle is mediated through H_1 receptors. H_1 receptor blocker mepyramine maleate did protect the histamine-induced bronchospasm and the resultant death but for a large dose of 15 mg/kg. In clinical practice also, several investigators have reported some beneficial effects with doses of antihistamines higher than those used for allergic rhinitis[53,54]; these effects were particularly noticeable when antihistamine was given intravenously[55]. However, the extract offered

the observed protection (40%) at the usual experimental dose-level. It would be interesting to study the effect of higher doses of the extract for it may offer still better degree of protection.

In the doses used, the extract did not produce any significant degree of suppression of the oedemagenic action of carrageenan in the rat hind limb paw nor of granulation tissue formation around the subcutaneously implanted cotton pellets. Apparently, therefore, the extract was devoid of anti-inflammatory effect since the classical anti-inflammatory agents, both of steroidal and nonsteroidal nature, antagonize the inflammation in these experimental models[56]. Caution is, however, required in the interpretation of the results of experimental study regarding the devoidance of anti-inflammatory effect of the extract to the clinical situation; the type of inflammation in the tracheobronchial musculature in patients might be of a different type than experimentally produced in the present study. Other models of inflammation need to be investigated.

Carrageenan induced oedema formation is mediated through the release of various mediators, locally, at the site of carrageenan injection in a sequential manner[57]. It is curious to note that though the extract effectively antagonized the release of mediators of anaphylaxis induced by the antigen from the sensitized lung and of histamine by compound 48/80 from the non-sensitized lung, it failed to alter the carrageenan-induced release of mediators of inflammation. This finding suggests that the extract exerts a specific effect on the release process in the bronchial tissue. It is, therefore, not unlikely that the extract might as well have some specific effect on the inflammatory process in the tracheobronchial system.

Oral administration of the extract did not produce any marked effect on the gastrointestinal motility as was observed by the travel rate of charcoal meal in mice. However, it significantly inhibited the carbachol-induced increase in the gastrointestinal motility. This finding, coupled with its effects on the isolated guinea pig ileum and

rabbit jejunum, do suggest that the extract possesses some anticholinergic effects. The maintenance of mild degree of airway tone in animals and man is mediated by parasympathetic cholinergic nerves[58,59]. Furthermore, inhalation of irritants will provoke bronchoconstriction in susceptible individuals which can be prevented by the prior administration of anticholinergic drug atropine and, accordingly, the role of parasympathetic nervous system in the maintenance of chronic airway obstruction has been emphasized[60]. At least part of the chronic airway obstruction in many perenially asthmatic children is a consequence of parasympathetically mediated bronchospasm[61]. It is, therefore, likely that the extract by exerting anticholinergic action, besides many diverse type of actions as discussed above, might produce a complimentary effect in irritants-provoked bronchoconstriction and, to some extent, in parasympathetically mediated chronic airway obstruction.

Altogether, the results of the present study may help to establish a scientific basis for the use of these plants, in crude form, in the diseases of tracheobronchial system or chronic airway obstruction. Briefly, the above mentioned findings suggest that the extract produces its beneficial effects by (i) exerting antihistaminic effect, (ii) causing smooth muscle relaxation, (iii) preventing the release of pharmacologically-active substances both under immunological and non-immunological conditions, (iv) inhibiting the antigen-antibody interaction, (v) preventing the hypersensitivity response, (vi) causing mast cell stabilization, (vii) reducing the histamine aerosol-induced bronchopasm, (viii) inhibiting the increase in capillary permeability and (ix) exerting alluded anticholinergic action.

SUMMARY

The crude aqueous extract of a herbal formula composed of nine plant ingredients, namely-dried fruits of *Vitis vinifera, Zizyphus vulgaris, Cordia latifolia, Malva sylvestris,* seeds of

Althaea officinalis, rhizome of *Glycyrrhiza glabra*, whole plants of *Lavandula stoechas* and *Adiantum capillus veneris* and flowers of *Viola odorata*, has been found to antagonise, in smaller doses, the spasmogenic effect of histamine, and, in larger doses, that of the other agonists on the isolated guinea pig ileum and of calcium chloride on the depolarized tissue. On rabbit jejunum, it produced relaxation and augmented the responses of isoprenaline and adrenaline. It inhibited the spasmogenic effects of 5-hydroxytryptamine and $PGF_1\alpha$ and $F_2\alpha$ on the rat fundus and of PGE_1 on rat uterus. It stimulated the guinea pig atria but in substimulant doses antagonized the effects of isoprenaline and histamine. It inhibited the Schultz-Dale reaction, reduced the release of pharmacologically-active substances from the lungs induced by the antigen or compound 48/80, appreciably stabilized the mast cells, inhibited the cutaneous hypersensitivity response as also the increase in cutaneous capillary permeability induced by histamine, 5-hydroxytryptamine and bradykinin, markedly suppressed the histamine aerosol-induced bronchospasm and inhibited the carbachol-induced increase in gastrointestinal motility. These findings convincingly support the clinical usefulness of these plants in the treatment of bronchial asthma and chronic bronchitis.

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TABLE I: EFFECT OF THE EXTRACT ON THE RELEASE OF MEDIATOR(S) FROM THE LUNG TISSUE, INDUCED BY COMPOUND 48/80 OR ANTIGEN, CALCULATED IN TERMS OF HISTAMINE (ug/gm).

Tissue	Mediators concentration Mean ± S.E.
Control lung from nonsensitized animal	0.43 ± 0.40
Lung from nonsensitized animal exposed to compound 48/80	3.58 ± 0.14
Lung from nonsensitized animal first exposed to the extract and then to compound 48/80	0.53 ± 0.13
Control lung from sensitized animal	1.36 ± 0.11
Lung from sensitized animal exposed to antigen	2.78 ± 0.13
Lung from sensitized animal first exposed to the extract and then to the antigen	1.86 ± 0.09

TABLE II: EFFECT OF THE EXTRACT ON ANTIGEN-INDUCED CUTANEOUS HYPERSENSITIVITY RESPONSE IN PARTIALLY ANAESTHETIZED GUINEA PIGS. THE ANIMALS WERE SENSITIZED WITH EGG WHITE 3-4 WEEKS BEFORE THE EXPERIMENT.

Drugs	Time in Hours					
	1/4	1/2	1	2	3	4
Normal saline	-	-	-	-	-	-
Egg albumin 5%	±	+ +	+ + + ±	+ + + +	+ + +	+ ±
Extract 100 mg	-	-	-	-	-	-
Extract 100 mg + Egg albumin 5%	-	-	-	-	-	-
Egg albumin 5% in extract-fed animal	-	-	±	+ ±	±	-

TABLE III: EFFECT OF THE EXTRACT AND MEPYRAMINE MALEATE ON THE INCREASE IN CUTANEOUS CAPILLARY PERMEABILITY INDUCED BY HISTAMINE.

Drugs	Time in Hours					
	1/4	1/2	1	2	3	4
Normal saline	-	-	-	-	-	-
Histamine 1 μg	±	+ +	+ + ±	+ ±	+ ±	±
Histamine 3 μg	+ +	+ + +	+ + +	+ + +	+ +	+
Histamine 10 μg	+ +	+ + +	+ + + +	+ + + +	+ + +	+ + ±
Extract 30 mg	-	±	±	-	-	-
Extract 30 mg + Histamine 1 μg	-	±	+	+	±	-
Extract 30 mg + Histamine 3 μg	-	+	+ ±	+	+	-
Extract 30 mg + Histamine 10 μg	-	+	+ ±	+ ±	+	±
Mepyramine 5 μg + Histamine 10 μg	-	+ +	+ ±	+	-	-
Mepyramine 10 μg + Histamine 10 μg	-	+	+	±	-	-
Mepyramine 15 μg + Histamine 10 μg	-	-	-	-	-	-

TABLE IV: EFFECT OF THE EXTRACT AND MEPYRAMINE MALEATE ON THE INCREASE IN CUTANEOUS CAPILLARY PERMEABILITY INDUCED BY BRADYKININ AND 5-HYDROXYTRYPTAMINE.

Drugs	Time in Hours					
	1/4	1/2	1	2	3	4
Normal saline	-	-	-	-	-	-
Bradykinin 1 μg	+ +	+ + + +	+ + + +	+ + + +	+ + + ±	+ + +
Extract 30 mg	-	-	-	-	-	-
Extract 30 mg + Bradykinin 1 μg	-	+ +	+ +	+ +	+ +	+ ±
Mepyramine 15 μg + Bradykinin 1 μg	+ + ±	+ + + +	+ + + +	+ + + +	+ + + +	+ + +
5-HT 1 μg	+	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
Extract 30 mg + 5 HT 1 μg	+	+ +	+ +	+ + ±	+ +	+
Mepyramine 15 μg + 5 HT 1 μg	+	+ + +	+ + + +	+ + + +	+ + + ±	+ + + ±

TABLE V: EFFECT OF THE EXTRACT AND MEPYRAMINE ON HISTAMINE AEROSOL - INDUCED BRONCHOSPASM IN GUINEA PIGS.

Group	Onset of convulsions in seconds	Number of animals died / Number of animals in the group	Per cent mortality
Control	68	5/5	100
Mepyramine 5 mg/kg	64	5/5	100
Mepyramine 15 mg/kg	No convulsion	0/5	0.0
Extract 20 gm/kg	128*	2/5	40

* Mean of three animals only.

TABLE VI: EFFECT OF THE EXTRACT ON MAST CELLS DEGRANULATION INDUCED BY COMPOUND 48/80.

Drugs	Per cent degranulation Mean ± S.E.	P value
Normal saline	4.0 ± 2.1	
C 48/80 10 μg/ml	38.0 ± 9.84	< 0.02
Extract 10 mg/ml + C 48/80 10 μg/ml	4.8 ± 2.78	

TABLE VII: EFFECT OF THE EXTRACT ON THE INTESTINAL MOTILITY OF MICE AS ASSESSED BY THE TRAVEL OF CHARCOAL MEAL.

Drugs	Dose/kg	Mean ± S.E. per cent length of small intestine travelled by charcoal meal
Distilled water	10 ml	64.0 ± 5.68
Extract, oral	10 gm	58.50 ± 5.20
Carbachol, i.p.	50 μg	74.37 ± 1.99
		P < .001
Extract, oral + Carbachol, i.p	10 gm + 50 μg	59.0 ± 2.32

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PHARMACOLOGICAL EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF CERTAIN MEDICINAL PLANTS

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INTRODUCTION

The plants *Withania somnifera* ("Asgandh Nagori; Aaksan"), *Merendra persica* ("Suranjan Sheerin"), *Pyrethrum indicum* ("Buzidaan") and *Alpinia galanga* ("Khulanjan Kabeer") have been mentioned as useful remedies for the treatment of diverse types of ailments in various ancient, as also in recent, texts. *Withania somnifera* roots have been used, both internally and externally, in rheumatism and as resolvent, tonic, alterative, aphrodisiac and uterotonic and as a prospective substitute for *Merendera persica* corm[1,2] which has been advocated by Al-Razi[3], Bu-Ali-Seena[4] and Ibn-al-Baytar[5] as an effective remedy for the treatment of rheumatism, gout, pain, constipation, sexual debility, muscular stiffness and rheumatic pain, and as alterative, aperient, laxative and in the diseases of liver and spleen[1], some of these actions being almost identical to that of *Pyrethrum idicum* root[1,2,4,5]. Similarly, *Alpinia galanga* rhizome has been recommended for rheumatism, catarrhal affections[2], visceral pain and as digestant and carminative[3,4,5] and also as aphrodisiac[1]. Critical overview of these texts, thus, revealed that, in common, the above-mentioned plants have been persistently, and particularly, recommended, over the centuries, for the treatment of rheumatoid syndrome. Indeed, a combina-

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tion of these plants has been found encouragingly effective in the treatment of clinical arthropathies in human subjects[6]. These informations, therefore, prompted us to undertake the present investigation to pharmacologically evaluate the anti-inflammatory and anti-arthritic activity of the above mentioned plants, individually and combinely, using certain relevant experimental models in order to vindicate the claims made by the ancient scholars and traditional healers.

MATERIALS AND METHODS

PLANT MATERIALS

Dried roots of *Withania somnifera* and *Pyrethrum indicum*, corm of *Merendra persica* and rhizome of *Alpinia galanga* were used in the present study.

EXTRACTION PROCEDURE

The plant materials were finely powdered with the help of a high speed grinding machine and the resultant power was sieved through a sieve (pore size 0.25 mm). The powder was then extracted with Sorensen buffer (pH 7.5); to each 100 gm of powder 2.5 litre of buffer was added. The extraction was done at 37°C for 5 hours during which period the mixture was continuously stirred (at a rate of 250 stirrings per minute) with the help of an electrical stirrer (Laboratory Supply Company, West Germany). Thereafter, the supernatant was filtered through Whatman filter paper No. 1. The filtrate, thus obtained, was stored at 4°C for experimental use.

DRUGS AND CHEMICALS

Carrageenan Type V, Freund's adjuvant, Bradykinin triacetate salt, Prostaglandins E_2 and $F_{2\alpha}$ (Sigma, U.S.A.), Histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate (B.D.H., England), Oxyphenbutazone (Tanderil; Geigy, Switzer-

land), Dexamethasone (Roussel, England) and anaesthetic ether (May & Baker, England).

DOSES

Unless otherwise mentioned, dexamethasone, oxyphenbutazone and the extracts were administered, orally, in doses of 5, 100 and 500 mg/kg body weight respectively.

ANIMALS

Inbred Wistar rats weighing between 150-300 gm of either sex were used in the study. The animals were maintained on standard pellet diet (86/A; Bugbrooke Mills, Northampton) and *ad lib* water.

INDUCTION OF RAT PAW OEDEMA BY CARRAGEENAN

(i) Assay of the oedemagenic action of carrageenan:

Forty rats, of either sex were randomly divided into groups of 10. Initial volume (ml) of the right hind paw upto a fixed mark at the level of lateral malleolus of each rat was recorded by means of a plethysmometer (Ugo Basile, Italy). Thereafter, the rats of separate groups were respectively injected, under the plantar aponeurosis, with 0.1, 0.25, 0.5 and 1.0% carrageenan solution (0.1 ml) in normal saline and the paw volume was again recorded after 3 hours. Percentage increases in paw thickness were calculated and compared for different groups separately. Allowance was made for the non-specific effect of the saline injection by substracting the value obtained for the contralateral saline-injected control paw from that of the test paw at the time of measurement[7]. The animals were then sacrificed at the end of 3 hr using excess of anesthetic ether, the plantar tissue collected and processed for histological examination.

(ii) Effect of the extracts on the temporal course of carrageenan-induced inflammatory process:

Oedema was induced[8] in the rats by single sub-plantar injection into the right hind paw of a solution of carrageenan (0.1 ml; 0.5%). Paw swelling was monitored as percentage change in dorso-ventral paw thickness which was measured immediately before, and at 1, 2, 3 hr after injection. The extracts or the comparable volume of distilled water were fed 1 hr before carrageenan.

(iii) Effect of the combined extract or oxyphenbutazone:

Acute oedema of the right hind paw was induced by 0.1 ml of 0.5% carrageenan injected under the plantar aponeurosis. The paw volumes were determined, 3 hr after carrageenan injection, both in the control and treated animals. The animals were pretreated with the test drugs, orally, 1 hr before carrageenan. In some experiments, the animals were pretreated for 24, 48, 72 and 96 hr at 24-hourly intervals, the last administration being 1 hr before carrageenan. Control animals received comparable volume of distilled water at similar occasions.

INDUCTION OF RAT PAW OEDEMA BY CERTAIN MEDIATORS OF INFLAMMATORY PROCESS

The oedema was produced[9] by intraplantar injection of 0.1 ml of histamine dihydrochloride (10 mg/ml), 5-hydroxytryptamine creatinine sulphate (0.05 mg/ml), bradykinin (0.05 mg/ml) and prostaglandin E_2 (0.005 mg/ml). The volume of oedema was determined at $^1/_2$, 1 and 2 hr after histamine or bradykinin, 1, 1 $^1/_2$ and 2 hr after 5-hydroxytryptamine and $^1/_4$, $^3/_4$, 1 $^1/_2$ and 2 hr after prostaglandin E_2 injection. The extracts, or comparable volume of distilled water, were fed 1 hr before injection of the phlogistic agents.

ESTIMATION OF HISTAMINE AND 5-HYDROXYTRYPTAMINE CONTENTS IN THE PLANTAR TISSUES

Rats from both the control and the extract treated groups were sacrificed 1 hr after the intraplantar injection of carrageenan, the plantar tissues collected and extracted in 10% trichloracetic acid for histamine and in acetone for 5-hydroxytryptamine by the method of Parrot and West[10]. The extracts were assayed on isolated guinea pig ileum for histamine[11] and on isolated rat fundus for 5-hydroxytryptamine[12,13].

COTTON PELLET GRANULOMA

Sterile cotton pellets 10 mg each were implanted[14] bilaterally in pectoral and groin regions of rats under ether anaesthesia. Cotton pellets were dissected out on the 7th day and wet and constant dry weights recorded. Drugs were administered once daily, orally, starting one day prior to the implantation of the pellets.

ADJUVANT-INDUCED ARTHRITIS

Freund's adjuvant (0.1 ml) was administered by single intraplantar injection into the right hind paw[15,25]. Drugs were fed once daily starting one day prior to adjuvant injection until day 14. The oedema volume was recorded immediately before and on days 1 (4 hr after adjuvant injection), 2, 3, 5, 7, 8, 10, 12, 14 and 15. The volume of the contralateral paw was also recorded simultaneously on all occasions. The primary response was characterised by the swelling of the injected paw and the delayed systemic or secondary response was characterised by the swelling of the contralateral hind paw, the forelimb paws and the appearance of nodules on the pinna and the tail.

CALCULATION OF THE INHIBITORY EFFECT OF TEST DRUGS

The anti-inflammatory activity has been expressed[16] as per cent inhibition of the oedema volume/weight of the granulomatous tissue which was calculated by the formula:- (1 - T/C) x 100, where T and C are the mean values of the drug-treated and the control groups respectively.

ISOLATED TISSUES

Pieces of rat fundus[12], guinea pig ileum, oestrogenized rat uterus, rabbit jejunum or frog rectus abdominis muscle[17] were suspended in 10 ml capacity isolated tissue bath containing Tyrode (37°C), Krebs (37°C), deJalon (31°C), or amphibian Ringer (room temperature - 21-22°C) solutions respectively; the solutions were continuously bubbled with air. The contractions were recorded on a smoked drum with a simple lever (magnification 1:7). For oestrogenization[18], adult female non-pregnant rats received oestradiol benzoate (0.1 mg) in 1 ml arachis oil intraperitoneally, daily, on 2 successive days. The animals were sacrificed 24 hr after the last injection and the uterine horns were used.

GROSS BEHAVIOURAL STUDIES

The effect of the extract was studied[19] on the gross behavioural changes in rats (200-250 gm) and mice (20-30 gm) of either sex randomly divided in groups of 10. The extract in varying doses (0.5, 1.0, 1.5 and 2.0 gm/kg) were administered *per os* and gross behavioural changes were recorded at 15, 30, 60, 120, 180 and 240 minutes.

LD_{50} STUDIES

Adult rats (150-300 gm) and mice (25-30 gm) of either sex were randomly divided in groups of 10. Graded doses of the extract (0.5, 1.0, 1.5 and 2.0 gm/kg) were fed to separate groups once and the mortality recorded for the next 8 days.

STATISTICAL ANALYSIS

The changes in the paw volume, amine concentrations or the granulomatous tissues were calculated as mean ± s.e.m. and the differences between groups evaluated statistically using Student's unpaired 't' test.

RESULTS

CARRAGEENAN-INDUCED OEDEMA OF THE RAT PAW

Assay of the oedemagenic action of carrageenan:

The results are presented in the Table I. The oedemagenic action of carrageenan was dose-dependent in that the degree of oedema formation was directly proportional to the concentration of carrageenan injected. Thus 0.1, 0.25, 0.5 and 1.0% concentrations of carrageenan (in 0.1 ml volume) increased the paw volume by 11.20, 26.39, 53.05 and 57.07% respectively; equal volume of normal saline increased the volume only by 0.3%. Histological examination revealed that upto a concentration of 0.5%, the degree of vascularity, exudation and oedema production was roughly related to the dose-increments; a decline was noticed with 1.0% concentration. Based on these observations, 0.5% concentration of carrageenan was used in all the experiments of the present study.

Effect of the extracts on temporal course of carrageenan-induced inflammatory process:

Table II summarizes the effect of different extracts as also of oxyphenbutazone and dexamethasone on oedemagenic action of carrageenan. In control group of animals, the mean initial paw volume was 1.44 ± .057 ml which increased to 2.03 ± .123, 2.34 ± .164, 2.57 ± .152 and 2.61 ± .155 ml (by 40.91, 62.80, 78.80 and 81.25%) at 1, 2, 3 and 4 hr respectively. At similar intervals in *Withania somnifera*-treated group, the paw volume (1.44 ± .038

ml) increased to 1.79 ± .034, 1.93 ± .074, 2.02 ± .130 and 2.07 ± .128 ml; thus it inhibited the carrageenan effect by 42.27 ($P < .05$), 46.66 ($P < .01$), 49.55 ($P < .001$) and 46.08 ($P < .01$) percent respectively. In *Merendra persica* treated group, the paw volume (1.53 ± .127 ml) increased to 1.84 ± .126, 2.01 ± .151, 2.14 ± .141 and 2.15 ± .131 ml at 1, 2, 3 and 4 hr respectively; hence, it inhibited the carrageenan effect by 49.06 ($P < .05$) 46.66 ($P < .01$), 46.01 ($P < .01$) and 46.55 ($P < .001$) percent respectively. *Alpinia galanga* failed to antagonize the oedemagenic effect of carrageenan. *Pyrethrum indicum* produced maximum inhibition (62.64%; $P < .01$) at the first hour which declined to 38.88 ($P < .05$) and 38.04 ($P < .01$) percent at the second and third hours; at the fourth hour, its inhibitory effect (22.4%) became insignificant. Combined extract from all the four plants produced comparatively better inhibitory effect wherein the initial paw volume (1.32 ± .042 ml) increased to 1.47 ± .029, 1.67 ± .048, 1.75 ± .093 and 1.82 ± .076 ml at 1, 2, 3 and 4 hr respectively; thus, it reduced the carrageenan effect by 72.23 ($P < .01$), 57.78 ($P < .001$), 58.66 ($P < .001$) and 53.39 ($P < .001$) percent at the respective intervals. Dexamethasone inhibited the carrageenan response by 72.83 ($P < .001$), 80.00 ($P < .001$), 73.45 ($P < .001$) and 74.13 ($P < .001$) percent at respective intervals. However, the effect of oxyphenbutazone was weaker, in that, it inhibited the carrageenan oedema by 32.08, 36.66, 32.74 and 27.01%, the last two figures being significant at $< .05$ level.

EFFECT OF THE COMBINED EXTRACT AND OXYPHENBUTAZONE IN RELATION TO THE INCREMENTS IN PRETREATMENT PERIODS

Results are presented in Table III. In 1hr pretreated group, carrageenan increased the paw volume from 1.70 ± .048 to 2.89 ± .076 ml in controls, from 1.59 ± .052 to 2.03 ± .061 ml in the extract-treated and from 1.64 ± .043 to 2.55 ± .058 ml in oxyphenbutazone-treated rats; thus, the extract inhibited the oedema by 46.93 (P < .001) and oxyphenbutazone by 28.10 (P < .05) percent. On increasing the duration of pretreatment to 24 hr, the effect of the extract remained almost unchanged but that of oxyphenbutazone markedly enhanced, the percent inhibition being 34.17 (P < .05). However, by increasing the duration of pretreatment further to 48, 72 and 96 hr, the extract proportionately and significantly (P > .001) inhibited the carrageenan-induced oedema by 62.07, 75.82 and 84.78% respectively; the respective values for oxyphenbutazone were 54.64, 89.32 and 78.63%.

EFFECT OF THE EXTRACTS ON HISTAMINE-INDUCED OEDEMA

Results are summarised in Table IV. Histamine increased the paw volume from 1.61 ± .04 to 2.58 ± .08, 2.31 ± .11, 2.22 ± .09 and 2.17 ± .09 ml after $^1/_2$, 1, 1 $^1/_2$ and 2 hr respectively. Thus, the maximum increase was at $^1/_2$ hr (60.24%) which sharply declined at 1 hr (43.47%) and thereafter gradually at 1 $^1/_2$ (37.80%) and 2 hr (35.1%). *Withania somnifera* and *Merendra persica* had no effect on the oedemagenic effect of histamine. However, *Alpinia galanga* and *Pyrethrum indicum* effectively and significantly antagonised the histamine response; the respective percent inhibitions were 49.48 (P > .01), 58.57 (P < .001) and 34.42 (P < .05) for *Alpinia galanga* and 70.10, 91.42 and 98.36 for *Pyrethrum indicum* (P < .001). The combined extract of the four plants significantly inhibited the histamine response, at the initial stages by 43.29 (P < .001) and

30.00 ($P < .01$) percent at $^1/_2$ and 1 hr respectively; at the later stage (1 $^1/_2$ hr) the degree of inhibition became insignificant (16.39%).

EFFECT OF THE EXTRACTS ON 5-HYDROXYTRYPTAMINE-INDUCED OEDEMA

The results are presented in Table V. In control group of animals, 5-hydroxytryptamine increased the paw volume from 1.49 ± .05 ml to 2.43 ± .11, 2.40 ± .11 and 2.35 ± .10 ml at 1, 1 $^1/_2$ and 2 hr respectively, the respective percent increases being 77.96, 69.34 and 68.17. *Withania somnifera* failed to significantly antagonise (13.82%) its effect at the first hour but subsequently inhibited ($P < .01$) it by 34.14 and 39.53% at 1 $^1/_2$ and 2 hr respectively. *Merendra persica* and *Alpinia galanga* were highly effective, in that, the former suppressed ($P < .01$ to $< .001$) the oedema by 38.29, 40.24 and 65.11% and the latter by 30.85, 41.46 and 53.48% at 1, 1$^1/_2$ and 2 hr respectively. However, like *Withania somnifera, Pyrethrum indicum* also did not inhibit the 5-hydroxytryptamine response at the initial stage (1 hr) but subsequently inhibited it by 31.70 ($P < .05$) and 60.46 ($P < .001$) percent at 1$^1/_2$ and 2 hr respectively. The combined extract effectively and significantly inhibited the oedema at all the three intervals by 24.46 ($P < .01$), 39.82 ($P < .001$) and 51.39 ($P < .001$) percent.

EFFECT OF THE EXTRACTS ON BRADYKININ-INDUCED OEDEMA

The results are summarised in table VI. In control group of animals, bradykinin increased the paw volume from 1.45 ± .04 ml to 1.91 ± .07, 1.95 ± .09 and 1.93 ± .09 ml at $^1/_2$, 1 and 1$^1/_2$ hr respectively, the respective percent increase being 31.50, 34.24 and 32.87. Whereas *Withania somnifera, Merendra persica* or *Pyrethrum indicum* failed to significantly antagonise the bradykinin response at the initial stage ($^1/_2$ hr), that of the *Alpinia galanga* highly, persistently and significantly inhibited ($P < .001$) it by 89.13, 96.00 and 97.91% at $^1/_2$, 1 and 1$^1/_2$ hr respectively. However,

the former three plants also antagonised ($P < .01$) the bradykinin response at subsequent stages (1 and $1\frac{1}{2}$ hr). The combined extract effectively and significantly ($P < .01$) inhibited the bradykinin response by 58.69, 46.00 and 52.08% at $^1/_2$, 1 and 1 $^1/_2$ hr respectively.

EFFECT OF THE EXTRACTS ON PROSTAGLANDIN E_2-INDUCED OEDEMA

The results are presented in Table VII. *Withania somnifera* markedly and significantly ($P < .001$) inhibited the prostaglandin response by 85.71, 83.33, 86.95 and 81.81 percent at 15, 45, 90 and 120 minutes respectively. Similarly, *Alpinia galanga* produced high degree ($P < .001$) of inhibition; the respective values were 82.14, 78.57, 95.65 and 81.18%. However, *Merendra persica*-induced inhibition was comparatively lesser, but significant, in that it inhibited, at the respective intervals, the prostaglandin-induced oedema by 51.65, 62.45, 60.28 and 59.43%. *Pyrethrum indicum* was entirely ineffective. The combined extract of all the four plants effectively and significantly ($P < .001$) reduced the prostaglandin effect by 57.14, 73.91, 69.56 and 86.36% at the respectivel intervals.

EFFECT OF THE COMBINED EXTRACT ON TISSUE HISTAMINE CONCENTRATION

The results are summarized in Table VIII. The concentration of histamine in the plantar tissue of the normal paw was estimated to be 70.70 ± 2.60 ug/gm which was markedly and significantly ($P > .001$) reduced to 44.58 ± 1.13 ug/gm in the carrageenan-injected paw. However, this reduction was significantly ($P > .001$) lower in the extract-treated animals; the percent reduction being 11.47 compared to 36.94 in the non-treated animals.

EFFECT OF THE COMBINED EXTRACT ON TISSUE 5-HYDROXYTRYPTAMINE CONCENTRATION

The results are presented in Table IX. Like histamine, carrageenan also reduced markedly and significantly (P > .001) the 5-hydroxytryptamine concentration from 174.31 ± 0.71 to 93.0 ± 0.37 ng/gm. However, this reduction was significantly (P > .001) lower in the extract-treated animals, the percent reduction being 20.83 compared to 46.64 in the non-treated animals.

EFFECT OF THE COMBINED EXTRACT, OXYPHENBUTAZONE OR DEXAMETHASONE ON COTTON PELLET-INDUCED GRANULATION TISSUE FORMATION

The results are presented in Table X. In the control group of animals, the wet and constant dry weights of the granulations tissue were 288.01 ± 8.47 and 66.73 ± 4.54 mg respectively. The respective weights were reduced significantly (P < .001) to 98.41 ± 5.33 (by 56.83%) and 29.28 ± 0.95 (by 56.12%) in extract-treated, to 91.78 ± 4.46 (by 59.75%) and 27.46 ± 1.48 (by 58.85%) in oxyphenbutazone-treated and to 85.65 ± 3.15 (by 62.46%) and 26.97 ± 1.62 (by 59.69%) in dexamethasone-treated (P > .001) groups.

EFFECT OF THE COMBINED EXTRACT, OXYPHENBUTAZONE OR DEXAMETHASONE ON FREUND'S ADJUVANT-INDUCED ARTHRITIS

The results are presented in Table XI. In control group of rats, subplantar injection of adjuvant increased the paw volume by 30.30% after 4 hr (day 1) which further increased gradually to 44.69, 52.27, 59.84 on days 2, 3 and 5 respectively. Thereafter, the raised volume started declining, reaching the second day value on day 7 (44.69%) and nearly that of the first day on day 8 (33.33%). However, from day 10 onwards the volume again increased gradually to 37.12, 40.15 and 43.12% on days 10, 12 and 14

respectively until the end of observation period on day 15 (44.31%). The combined extract of the four plants markedly and significantly reduced the adjuvant response by 65.0% at the 4th hr and by 37.28, 43.47 and 34.17% on days 2, 3 and 5 respectively; from day 10 onwards the reduction was more marked and highly significant, the percent reduction being 48.97, 47.16, 52.63 and 56.89 on days 10, 12, 14 and 15 respectively. The effect of oxyphenbutazone was almost similar to that of the combined extract. However, the effect of dexamethasone was greater and significance higher than oxyphenbutazone or the extract, in that, dexamethasone-influenced inhibition ranged between 72.5 and 79.66% through the course of observation period except on days 3 and 5 when the reduction was 65.29 and 54.43% respectively.

The paw volume of the contralateral limb was also recorded on similar occasions. In control group of animals, the increases in the volume ranged between 18.23 to 27.34% from days 3 to 15. The combined extract as also oxyphenbutazone reduced the increase (range by 59.83-68.39%). Dexamethasone, however, inhibited it by 89.08 to 97.64%. The delayed systemic response of the secondary phase was also characterised by the swelling of the front paws and the appearance of nodules on the pinna and the tail. On gross assessment of the signs, control animals scored + + + ± grades while those of the extract, oxyphenbutazone and dexamethasone-treated groups scored + ±, + and ± grades respectively.

EFFECT OF THE EXTRACT ON ISOLATED TISSUES

Guinea pig ileum:- The combined extract (0.3-3.0 mg/ml) induced dose-dependent, reversible and reproducible contractions which were abolished after atropinization of the tissue. On the atropinized tissue, the *Pyrethrum indicum* extract reversibly inhibited the spasmogenic effect of histamine dihydrochloride (.02 ug/ ml) by 18.75, 43.75 and 72.5% at a dose of 0.3, 0.5, 1.0 mg/ml

respectively. Almost similar degree of inhibition was produced by the combined extract at 0.6, 1 and 2 mg/ml doses. The degree of inhibition could be overcome by increasing the dose of histamine. Thus the extracts-induced antagonism was roughly dose-dependent, reversible, consistent and competitive.

Rabbit jejunum:- The extract of *Withania somnifera* (upto1 mg/ml) did not produce any marked effect on the pendular movements nor altered the acetycholine response. On the other hand, extracts (0.6 mg/ml) of *Merendera persica, Alpinia galanga* or *Pyrethrum indicum* inhibited the pendular movements, and relaxed the tissue, thereafter the latter two extracts enhanced the pendular movements. The combined extract (2 mg/ml) of the four plants exerted greater effect than either of the extract alone, in that it pronouncedly inhibited the pendular movements, markedly relaxed the tissue followed by the increase in the tone and movements of the tissue.

Further, all the extracts (except that of *Withania somnifera*) separately potentiated the spasmogenic effect of acetycholine (0.05 ug/ml) by 55.55 - 66.66% at a dose of 0.6 mg/ml. Interestingly, however, the combined extract of all the four plants produced much greater degree of potentiation (116.66%) than any of the extracts alone. The extracts, which augmented the acetylcholine response, delayed the inactivation of acetylcholine (.5 ug) incubated with 0.2 ml rabbit serum. These extracts had no effect on the carbachol (.1 ug/ml)-induced contractions.

Rat fundus:- The combined extract produced dose dependent, reversible and reproducible contractile effect. However, this effect was abolished on atropinization. On the atropinized tissue, the extract produced dose-dependent relaxation. Whereas *Pyrethrum indicum* (.6 mg/ml) extract did not alter the 5-HT (.03 ug/ml) response, those of *Withania somnifera, Alpinia galanga* or *Merendra persica* (.6 mg/ml) inhibited it by 21.42, 42.85 or 28.57% respectively. However, the combined extract of the four plants (2 mg/ml)

inhibited the 5-HT-induced contraction by 53.24% which was greater than any of the extracts alone.

On the other hand, on tissues atropinized with usual doses of atropine (1 mg/litre), the extracts (.3 mg/ml) of *Withania somnifera, Merendra persica* or *Alpinia galanga* markedly potentiated, by about 50-80%, the spasmogenic effects of prostaglandin E_2 and $F_2\alpha$. However, on the highly atropinized tissues (10 mg atropine/litre), the extracts from the latter two plants inhibited the prostaglandin E_2 response by 49.83 and 61.66% respectively. The combined extract (2 mg/ml) inhibited the same by 73.40%.

Rat uterus: On the non-atropinized tissues, the extract produced pronounced contractile effect which could be repeatedly elicited without any sign of tachyphylaxis. However, on the atropinized tissues, the extracts failed to produce direct stimulant effect; rather the extracts (.1 mg/ml) of *Merendra persica* or *Alpinia galanga*, but not of *Withania somnifera* or *Pyrenthrum indicum*, completely inhibited the effect of prostaglandin E_2. The combined extract of the 4 plants also produced similar degree of inhibition.

Frog rectus: The combined extract did not exert any direct effect of its own. However, it (3 mg/ml) markedly potentiated the spasmogenic effect of acetycholine (.5 ug/ml) by about 75%. The potentiation persisted even after the extract was washed out; the tissue regained the normal response only after repeated exposure to, and washes of, acetylcholine. Further, it prevented the hydrolysis of acetylcholine (0.5 ug) when incubated with rabbit serum (0.2 ml). However, it did not alter the carbachol (0.5 ug/ml)-induced contractions.

GROSS BEHAVIOURAL CHANGES

The mice receiving the combined extract (1-2 gm/kg) became hyperactive and mildly aggressive together with itching in the pelvic and scrotal regions; the symptoms appeared within 30 min and persisted until 3 hr. During this period, animals frequently stood on

the hind limb, sat on the hind quarter and often mounted on the fellow companions. In one group of mice, the extract was administered in promethazine (5 mg/kg) pretreated animals to study whether the itching was due to the release of endogenous histamine. Interestingly, however, this group of animals became highly depressed until 4 hr observation period. Thereafter, the animals became fully active. In the rat also, the extract (1-2 gm/kg) produced hyperactivity and itching of the hind quarter and scrotal regions.

LD_{50} STUDIES

The extract did not exert any lethal effect upto a dose of 2 gm/kg in rats or mice until 8 days of observation period.

DISCUSSION

Carrageenan, a mixture of polysacchrides composed of sulfated galactose obtained from Irish sea moss *Chondrus crispus*, has been regarded as an ideal phlogistic agent because its inflammation is inhibited by non-toxic doses of all clinically-effective anti-inflammatory drugs[20]. And, over the years, carrageenan-induced paw oedema in rats had become most extensively used inflammation model for the screening of anti-inflammatory agents[21] since it was first introduced as an assay method for such agents[22]. Further, carrageenan is devoid of antigenic properties; thus, its effect depends upon the stimulation of a local inflammatory response[23]. However, different samples of carrageenan show considerable variation in their inflammatory potency; therefore, the inflammatory dose needs to be worked out for each carrageenan sample[24]. Accordingly, the potency of the carrageenan sample used in the present study was assayed; in that, graded doses of carrageenan ranging from 0.1 to 1% was tested for the oedemagenic response. It was observed that in 0.1, 0.25 and 0.5% concentrations, carrageenan induced a clear dose-dependent oedemagenic response; how-

ever, the effect of 1% did not follow the course of linearity in that its response did not differ much from that of 0.5%. As such, in our experiments 0.5% concentration was considered as optimal and was accordingly used in the present study. Indeed, the oedemagenic response of this concentration was roughly comparable to that obtained with 1% by other workers[7,9,16,26].

The subtle kaleidoscopic cascade of integrated events which constitute the reaction of tissue to phlogistic agent over a variable time scale is obviously mediated, modulated and orchestrated by a wide variety of chemical messengers which influence and determine vascular, extracellular and cellular sequellae, the prolixity of which follow a definite time-course of underlying processes. According to the earlier study[27], after the injection of carrageenan the first response is an increase in the output of histamine and 5-hydroxytryptamine ($1^1/_2$ hr), this is followed by bradykinin (2 hr) and prostaglandins release begins at about the third hr, corresponding to the amine, kinin and prostaglandin phases of inflammation. Accordingly, the effect of individual extracts was studied at hourly intervals until the 4th hr to cover-up different phases of inflammatory process. Our results indicate that *Withania somnifera* and *Merendra persica* significantly inhibited the carrageenan response through the entire 4 hr observation period, *Alpinia galanga* was entirely ineffective and *Pyrethrum indicum* was effective only upto the third hr. Based on these observations, the effect of these plants was studied separately on the oedema induced by direct injections of histamine, 5-hydroxytryptamine, bradykinin or prostaglandin E_2.

On histamine-induced oedema, *Withania somnifera* had no effect, *Merendra persica* had mild and *Alpinia galanga* and *Pyrethrum indicum* had pronounced suppressive effect. Thus, the inhibition of carrageenan response at the first hour by *Withania somnifera* and *Merendra persica* does not appear to be due to the

blockade of histamine (H_1) receptors. However, denial of a role of histamine, in this situation, must now be reconsidered in-view of the recent discovery[7] that H_2 receptor antagonists partially suppress carrageenan-induced oedema which has revealed a completely different view of the role of histamine in this process. Possibly, therefore, *Withania somnifera* and *Merendra persica* suppress the early phase of inflammation by blocking the H_2 receptors which get specifically activated under the influence of carrageenan. Additionally, these extracts may also inhibit the release of endogenous histamine locally at the site of carrageenan injection. Although, *Alpinia galanga* failed to significantly suppress the carrageean-induced oedema, it markedly inhibited that produced by histamine. Classical antihistamine-mepyramine suppresses the histamine-induced oedema[28] but not that induced by carrageenan[27]. This, therefore, indicates that *Alpinia galanga* exhibits actions similar to that of H_1 receptor blocker. *Pyrethrum indicum* suppressed the carrageenan response maximally at the first hour which sharply declined at the second and third hours and reached to insignificant levels at the fourth hour; however, its inhibitory effect against histamine-induced oedema was in the increasing order with the passage of time. Possibly, this plant has dual action both on the H_1 and H_2 receptors. Precisely, therefore, the results suggest that of the four plants, two (*Withania somnifera* and *Merendra persica*) act as H_2 blockers, *Alpinia galanga* as H_1 blocker and *Pyrethrum indicum* as blocker for both the H_1 and H_2 receptors. Notably, however, whereas *Pyrethrum indicum* inhibited, *in vitro*, the spasmogenic effect of histamine on isolated guinea pig ileum, *Alpinia galanga* did not; thus, presumably, *Alpinia galanga* undergoes certain metabolic changes *in vivo* and the resultant metabolites exert inhibitory effect on histamine-induced oedema. The combined extract from all the four plants exhibited a synergistic effect in that the suppression of the first hour response of carrageenan was greater than any of the

plants individually. However, its effect against histamine-induced oedema, as compared to *Alpinia galanga*, was short-lived but, during that period, was more pronounced. This could be due to certain form of interaction taking place between the four plants at the latter stages of their combined effect. Indeed, the first hour suppression of carrageenan-induced oedema by the combined extract was comparable to that produced by dexamethasone and was much higher than that by oxyphenbutazone.

On the 5-hydroxytryptamine-induced oedema, whereas *Merendra persica* and *Alpinia galanga* had pronounced suppressive effect just from the beginning of the observation period, the effect of *Withania somnifera* and *Pyrethrum indicum* discerned 30 min later. Possibly, the latter two plants undergo certain metabolic changes in the body before they exert anti-5-hydroxytryptamine effect. However, this assumption may not be necessarily unquestionable since on the isolated rat fundus, *Withania somnifera* produced direct antagonistic effect against 5-hydroxytryptamine-induced contractions. Further studies may unravel the possible mechanism. Although *Alpinia galanga* failed to significantly suppress carrageenan-induced oedema, it markedly inhibited that produced by 5-hydroxytryptamine, as it had done for that by histamine. Apparently, this plant exerts similar action against both histamine and 5-hydroxytryptamine. Moreover, *Alpinia galanga* also inhibited the spasmogenic effect of 5-hydroxytryptamine on rat fundus. Therefore, it may be inferred, at this stage, that this plant has the ability to block the 5-hydroxytryptamine receptors. The combined extract of all the four plants significantly suppressed the 5-hydroxytryptamine oedema from the beginning until the end of observation period, thus balancing the delayed phase of the onset of the effects of *Withania somnifera* and *Pyrethrum indicum*.

Alpinia galanga almost completely suppressed the bradykinin-induced oedema; the effect being discernible from the beginning

until the end of observation period. However, the remaining three plants produced their effect 30 min later. Apparently, therefore, these plants undergo certain metabolic changes before they exert their action against bradykinin. The combined extract of the four plants was comparatively less effective in suppressing the bradykinin effect than *Alpinia galanga*. Possible interaction between the four plants needs to be worked out. On the other hand, *Alpinia galanga* failed to suppress the carrageenan-induced inflammation whereas the other plants did. This, therefore, indicates that *Alpinia galanga* acts directly on the bradykinin receptive sites and does not affect the release of kinins.

The prostaglandin E_2-induced oedema was suppressed highly effectively by *Withania somnifera* and *Alpinia galanga* followed by *Merendra persica*; *Pyrethrum indicum* was entirely ineffective. Curiously, however, *Withania somnifera* failed to antagonise the prostaglandin E_2-induced contractions of the rat uterus; thus, its action on the inflammatory site is, apparently, specific. The effect of *Alpinia galanga* appears to be again complex since, on the one hand, it failed to suppress carrageenan-induced oedema, on the other hand, it effectively inhibited the prostaglandin response both in the paw and on the uterine tissue. These findings, therefore, suggest that *Alpinia galanga* blocks the direct activation of prostaglandin receptive sites and does not influence the prostaglandin release process. *Merendra persica* inhibited the carrageenan-induced oedema, the prostaglandin-induced oedema and also the prostaglandin-induced contraction of the uterine tissue. Thus, this plant appears to act both by preventing the release of prostaglandins and by blocking its receptors. *Pyrethrum indicum* failed to inhibit both the oedemagenic and the spasmogenic actions of prostaglandin but suppressed the initial stages of carrageenan-induced oedema. Thus, its effect appears to be limited only to the amine and kinin phases of inflammation. The combined extract of the four plants suppressed

the oedemagenic effect of prostaglandin as it did that of carrageenan and also blocked the spasmogenic effect of prostaglandin on the rat uterus. Thus, the combined extract possibly exerts its effect by preventing the release of prostaglandins and also by inhibiting its receptors.

On increasing the duration of pretreatment periods, combined extract produced better suppression of carrageenan-induced inflammation. Apparently, the combined extract undergoes slow releasable metabolic changes and the released metabolites exert their fullest effect with the passage of time. The other possibility could be that the extract exerts or modifies some components of inflamamtory system or oedema formation such as the leukocytes, blood enzymes, proteins, and precursors etc. involved in activation, release and inactivation of mediators or modulators in a slow, but effective manner. Interestingly, the course of efficacy of the extract was almost comparable to that of oxyphenbutazone.

Histamine is released locally at the site of carrageenan injection[27,29]. In the present study also significant depletion of tissue histamine has been observed. The combined extract significantly prevented the carrageenan-initiated release of histamine. Furthermore, it also prevented the local release of tissue 5-hydroxytryptamine. The modest role of 5-hydroxytryptamine in early phases of acute inflammation has been recognised[15,27]. Evidently, therefore, the combined extract suppresses, at least the early phases of carrageenan-induced acute inflammation by preventing the release of both histamine and 5-hydroxytryptamine; additionally, however, it also influences, as discussed in the preceding paragrpahs, the activation of respective receptors.

In the present study, the combined extract also prevented, significantly, the formation of granulation tissue. The cotton pellet granuloma assay is, perhaps, the most widely used method for assessing the effect of drugs on different phases of inflammation.

Three phases of inflammatory response to subcutaneous implantation of pellets has been described[30]. The first, short-lasting phase, of a few hours duration, is characterized by the imbibition of the pellet with fluid of low protein content. In the second phase, lasting for 2-3 days, exudation of fluid containing protein is typical. The third phase is characterized by the appearance of collagen in granuloma, preceded by mucopolysacchride synthesis, and accompanied by the greatest increase in the number of fibroblasts. Admittedly, the cotton pellet granuloma assay is relatively good test for evaluating the anti-inflammatory activity of steroids but is less satisfactory for non-steroidal compounds[31.32]. However, in our studies not much difference was observed between the animals treated with dexamethasone and those with oxyphenbutazone or combined extract. Nonetheless, certain immuno-suppressive drugs are also capable of inhibiting the granuloma formation, apparently by an intrinsic anti-inflammatory activity[33,34]. It would, therefore, be interesting to study whether this extract possesses immunosuppressive activity.

Several different types of experimental arthritis can be induced in animals. Their similarity to human disease is a matter of debate. However, not entirely unsuccessful attempts have been made to devise animal models of the various rheumatoid diseases. Adjuvant-induced arthritis in rat is probably the best and most widely used of these models employed in the screening programs for anti-inflammatory drugs[15]. Indeed, in the present study, the combined extract of the four plants significantly inhibited the adjuvant-induced arthritis in rats and its effect was almost parallel to that produced by oxyphenbutazone. It has been suggested that adjuvant-induced arthritis constitutes a delayed hyper-sensitivity response to mycobacterial antigens which is evidenced by the fact that the disease can be transmitted to normal animals by sensitized lymphocytes from afflicted animals, the disease can be prevented by excision of lymph nodes draining the side of adjuvant injection and

immunity in animals exposed to tolerogenic doses of adjuvant early in life[35,36,37,38]. The severity of the disorder are known to be modified by steroidal and non-steroidal anti-inflammatory drugs[15]. Thus, the effect of the combined extract in this model is of more relevance to the clinical situation. However, the arthritic syndrome can be alleviated or prevented by lymphocytotoxic drugs and antilymphocyte globulin[39] and inhibited through immunosuppression by antigenic competition[40,41,42]. It should, therefore, be interesting to study whether this extract possess immunosuppressive activity since it also inhibited in the granulation tissue formation.

Altogether, our results may help to establish a scientific basis for the use of these plants, in crude form, as an anti-inflammatory and antiarthritic remedy. The inhibition of the acute 3 hr swelling reflects the effectiveness of the combined extract on the acute inflammatory response. We also obtained evidence that the extract not only prevents oedema but is of comparable efficacy to oxyphebutazone in inhibiting the development of granulation tissue and the adjuvant-induced arthritis. These findings might prompt the view that, pharmacologically speaking, this extract represents another example of non-steroidal anti-inflammatory agent. However, further studies are required to elucidate the activity of the extract with respect to its effect on (i) enzymes necessary for the synthesis and release of mediators, (ii) complement levels and its activation, (iii) process of mononuclear cell exudation etc. in order to understand the mechanism by which extract interferes with various inflammatory and immunopathological reactions. Further, anti-inflammatory as distinct from immunosuppressive activity has been described in several models of experimental inflammation for immunosuppressive agents[15] such as mercaptopurine, methotrexates, cyclophosphamide, chlorambucil and actinomycin-D. These drugs mainly act by preventing the participation of macrophages in

delayed hypersensitivity and their infiltration into an inflammatory site. Similarly, a number of anti-inflammatory agents such as steroids, aspirin-like drugs, gold salts and pharmacological doses of oestrogen etc. have been shown to interfere with the immunopathological and inflammatory reactions such as adjuvant-induced arthritis. In view of these, it would be interesting to extend such studies on the extract as an immunosuppressive agent and elucidate its role in various immunopathological reactions as well as in experimental tumors in animal models.

SUMMARY

The crude aqueous extracts of the dried roots of *Withania somnifera* and *Pyrethrum indicum*, corm of *Merendra persica* and rhizome of *Alpinia galanga* were screened for their anti-inflammatory activity, either individually or combined, against the oedemagenic action of carrageenan and certain pharmacological mediators (histamine, 5-hydroxytrypotamine, bradykinin and prostaglandin E_2), the cotton pellet-induced granulation tissue formation and the adjuvant-induced arthritis in rats. Excepting *Alpinia galanga*, the remaining three plants significantly inhibited the carrageenan response. However, *Alpinia galanga* effectively antagonized the response of mediators; the effect of other plants and of their combined extract against mediators was variable. The combined extract of four plants produced highly pronounced suppressive effect against carrageenan-induced oedema and the carrageenan initiated release of histamine and 5-hydroxytryptamine. The combined extract also inhibited the cotton pellet induced granulation tissue formation and the adjuvant-induced arthritis. Its effect in these models was comparable to that of oxyphenbutazone. Possible mechanism of action has been discussed. The findings convincingly support the clinical usefulness of these plants in the treatment of rheumatoid syndrome and other inflammatory disorders.

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TABLE I: ASSAY OF THE OEDEMAGENIC ACTION OF CARRAGEENAN IN THE RAT HIND PAW (n = 10)

Group	Paw volume (ml)		% increase
	0 hr	3 hr	
Saline	1.65 ±.17	1.65 ±.17	0.30
Carrageenan 0.1%	1.71 ±.16	1.91 ±.17	11.20
0.25%	1.65 ±.18	2.09 ±.17	26.39
0.5%	1.59 ±.16	2.43 ±.16	53.05
1.0%	1.69 ±.17	2.66 ±.18	57.07

TABLE II: EFFECT OF THE EXTRACTS, DEXAMETHASONE OR OXYPHENBUTAZONE ON THE TEMPORAL COURSE OF CARRAGEENAN-INDUCED INFLAMMATORY PROCESS.

Group	Number of animals	Percent inhibition of swelling			
		HOURS			
		1	2	3	4
Control	12	-	-	-	-
Withania somnifera	9	42.27*	46.66**	49.55***	46.08**
Merendra persica	11	49.06*	46.66**	46.01**	46.55***
Alpinia galanga	10	30.39	30.00	23.89	16.37
Pyrethrum indicum	10	62.64**	38.88*	38.04**	22.4
Combined extract	11	72.23**	57.78**	58.66***	53.39***
Dexamethasone	10	72.83***	80.00***	73.45****	74.13****
Oxyphenbutazone	8	32.08	36.66	32.74*	27.01*

* $P < .05$; ** $P < .01$; *** $P < .001$; **** $P > .001$

TABLE III: INHIBITION BY THE COMBINED EXTRACT OR PHENYLBUTAZONE ON CARRAGEENAN - INDUCED INFLAMMATION. RATS WERE PRETREATED FOR VARYING PERIODS. NUMBER OF ANIMALS IN EACH GROUP ARE GIVEN IN THE PARENTHESES.

Pretreatment duration (hr)	Control Oedema %	Percent inhibition of swelling	
		Extract	Oxyphenbutazone
1	70.2 (16)	46.93*** (9)	28.10* (9)
24	68.4 (8)	47.60** (9)	34.17* (6)
48	73.5 (9)	62.07*** (9)	54.64*** (10)
72	70.4 (7)	75.82*** (9)	89.32*** (10)
96	66.0 (7)	84.78*** (10)	78.63*** (8)

Control values show percent increases in paw volume.

* $P < .05$; $P^{**} < .01$; $P^{***} > .001$

TABLE IV: EFFECT OF THE EXTRACTS ON HISTAMINE-INDUCED OEDEMA OF THE RAT HIND PAW.

Groups	Number of animals	Percent inhibition of swelling		
		HOURS		
		$^1/_2$	1	$1^1/_2$
Control	16	-	-	-
Withania somnifera	15	6.18	1.42	4.91
Merendra persica	16	8.55	8.53	6.52
Alpinia galanga	16	49.48**	58.57***	34.42*
Pyrethrum indicum	14	70.10****	91.42****	98.36****
Combined extract	18	43.29***	30.00**	16.39

*P < .05; **P > .01; *** P < .001; **** P > .001

TABLE V: EFFECT OF THE EXTRACTS ON 5-HYDROXYTRYPTAMINE-INDUCED OEDEMA OF THE RAT HIND PAW

Group	Number of animals	Percent inhibition of swelling		
		HOURS		
		1	$1^1/_2$	2
Control	15	-	-	-
Withania somnifera	14	13.82	34.14**	39.53**
Merendra persica	14	38.29**	40.24**	65.11***
Alpinia galanga	12	30.85**	41.46**	53.48***
Pyrethrum indicum	11	20.21	31.70*	60.46***
Combined extract	15	24.46**	39.82***	51.39***

*P < .05; ** P > .01; *** P < .001

TABLE VI: EFFECT OF THE EXTRACTS ON BRADYKININ-INDUCED OEDEMA OF THE RAT HIND PAW

Groups	Number of animals	Percent inhibition of swelling		
		HOURS		
		1/2	1	1 1/2
Control	12	-	-	-
Withania somnifera	10	2.17	48.00*	50.00*
Merendra persica	10	39.13	56.00*	66.66*
Alpinia galanga	13	89.13**	96.00**	97.91**
Pyrethrum indicum	9	26.08	52.00*	58.33*
Combined extract	12	58.69*	46.00*	52.08*

$*P < .01$; $**P < .001$

TABLE VII: INHIBITION OF PROSTAGLANDIN E_2 - INDUCED OEDEMA BY THE EXTRACTS

Groups	Number of animals	Percent inhibition of swelling			
		MINUTES			
		15	45	90	120
Control	10	-	-	-	-
Withania somnifera	9	85.71**	83.33**	86.95**	81.81**
Merendra persica	13	51.65*	62.45**	60.28**	59.43**
Alpinia galanga	11	82.14**	78.57**	95.65*	81.81*
Pyrethrum indicum	10	10.71	7.12	4.34	4.54
Combined extract	12	57.14*	73.91**	69.56**	86.36**

$*P < .01$; $**P < .001$

TABLE VIII: EFFECT OF THE COMBINED EXTRACT ON HISTAMINE (ug/gm) CONCENTRATION IN RAT PLANTAR TISSUES LOCALLY INJECTED WITH CARRAGEENAN. TWELVE TISSUES IN EACH EXPERIMENT.

<table>
<tr><th>Group</th><th>Mean ± s.e.m. concentration</th><th>Percent reduction</th><th>P value</th></tr>
<tr><td>Normal paw</td><td>70.70
± 2.60</td><td>-</td><td>-</td></tr>
<tr><td>Carrageenan treated paw</td><td>44.58**
± 1.13</td><td>36.94</td><td rowspan="2">} > .001</td></tr>
<tr><td>Paw from the extract-fed and carrageenan-injected animals</td><td>62.59*
± 0.83</td><td>11.47</td></tr>
</table>

*P < .001; **P < .001

TABLE IX: EFFECT OF THE COMBINED EXTRACT ON 5-HYDROXYTRYPTAMINE (ng/gm) CONCENTRATION IN RAT PLANTAR TISSUES LOCALLY INJECTED WITH CARRAGEENAN. TWELVE TISSUES IN EACH GROUP.

<table>
<tr><th>Group</th><th>Mean ± s.e.m. concentration</th><th>Percent reduction</th><th>P value</th></tr>
<tr><td>Normal paw</td><td>174.31
± 0.71</td><td>-</td><td></td></tr>
<tr><td>Carrageenan-treated paw</td><td>93.00*
± 0.37</td><td>46.64</td><td rowspan="2">} > .001</td></tr>
<tr><td>Paw from the extract-fed and carrageenan-injected animals</td><td>137.06*
± 1.60</td><td>20.83</td></tr>
</table>

*P > .001

TABLE X: EFFECT OF THE COMBINED EXTRACT, OXYPHENBUTAZONE OR DEXAMETHASONE ON COTTON PELLET - INDUCED GRANULATION TISSUE FORMATION.

Group	Number of animals	Mean ± s.e.m. weight (mg)		Percent reduction	
		Wet	Dry	Wet	Dry
Control	14	288.01 ± 8.47	66.73 ± 4.54	-	-
Combined extract	15	98.41 ± 5.33	29.28 ± 0.95	56.83*	56.12*
Oxyphenbutazone	10	91.78 ± 4.46	27.46 ± 1.48	59.75*	58.85*
Dexamethasone	12	85.65 ± 3.15	26.97 ± 1.62	62.46**	59.69**

* $P < .001$; ** $P > .001$

TABLE XI: EFFECT OF THE COMBINED EXTRACT, OXYPHENBUTAZONE OR DEXAMETHASONE ON ADJUVANT-INDUCED ARTHRITIS (n = 10)

Group	DAYS — Mean ± s.e.m. volume of right hind paw (% inhibition Vs. control)										
	0	1	2	3	5	7	8	10	12	14	15
Control	1.32 ± .03	1.72 ± .03	1.91 ± .04	2.01 ± .04	2.11 ± .03	1.91 ± .03	1.76 ± .03	1.81 ± .04	1.85 ± .05	1.89 ± .07	1.90 ± .05
Combined extract	1.34 ± .03	1.48 ± .04 (65.00) ****	1.71 ± .03 (37.28) **	1.73 ± .04 (43.47) ***	1.86 ± .03 (34.17) ***	1.73 ± .04 (33.89) ***	1.62 ± .05 (36.36) *	1.59 ± .04 (48.97) *	1.62 ± .04 (47.16) ***	1.61 ± .04 (52.63) ***	1.59 ± .04 (56.89) ***
Oxyphenbutazone	1.32 ± .03	1.47 ± .03 (62.5) ****	1.70 ± .03 (35.59) **	1.68 ± .04 (47.82) ***	1.87 ± .04 (30.37) ***	1.72 ± .04 (32.20) **	1.63 ± .03 (29.54) *	1.56 ± .04 (51.02) ***	1.60 ± .04 (47.16) ***	1.62 ± .03 (47.36) ***	1.58 ± .04 (55.17) ***
Dexamethasone****	1.38 ± .04	1.49 ± .02 (72.5)	1.50 ± .03 (79.66)	1.62 ± .05 (65.29)	1.74 ± .04 (54.43)	1.51 ± .03 (77.96)	1.48 ± .05 (77.27)	1.51 ± .04 (77.46)	1.51 ± .03 (75.47)	1.51 ± .03 (77.19)	1.51 ± .02 (77.58)

* $P < .05$; ** $P < .01$; *** $P < .001$; **** $P > .001$

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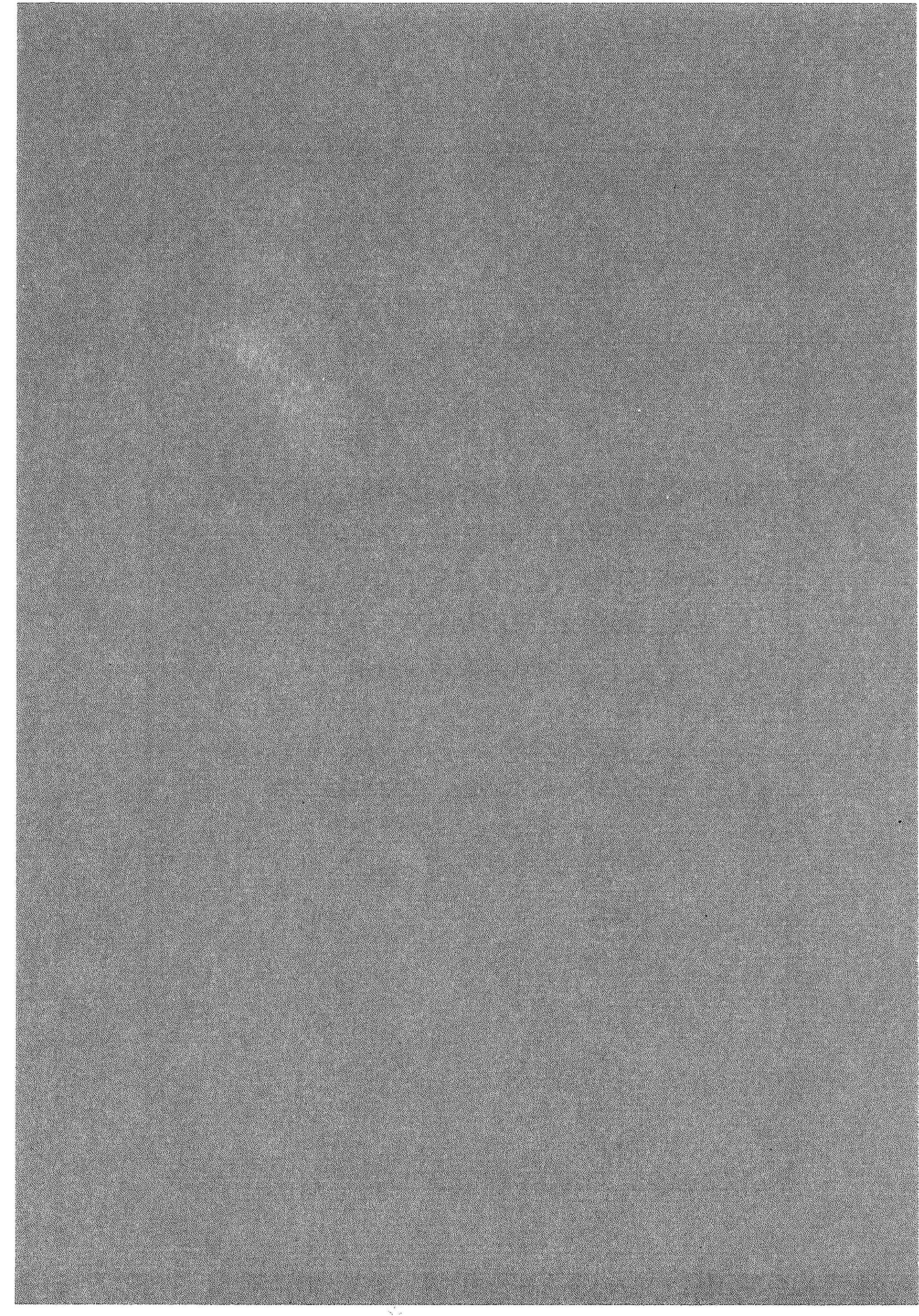
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EFFECT OF CERTAIN MEDICINAL PLANTS CLINICALLY USED IN RHEUMATOID ARTHRITIS ON EXPERIMENTALLY INDUCED ULCERS

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Abstract

Earlier studies revealed that an aqueous extract of a herbal formulation (RA) consisting of dried roots of *Withania somnifera* Linn, rhizome of *Alpinia galanga* Wild, corm of *Merendra persica* Linn and roots of *Pyrethrum indicum* DC exhibited significant anti-inflammatory activity in various experimental inflammatory models and was also devoid of any teratogenic and mutagenic potentials in mice. The present study was designed to investigate the effect of RA extract on experimentally induced ulcers in laboratory animals. Acute and chronic oral treatment of RA extract up to a period of 1 to 5 months in the dose levels of 400 and 800 mg/rat/day along with the drinking water (20 and 40 mg/ml) did not exhibit any abnormality both in the cardiac and pyloric portion of the stomach in Albino rats of either sex compared to controls. RA extract (0.5 g/kg) exhibited protection against the ulcers induced by pyloric ligation. The percentage reduction in ulcer score compared to control was 45.6 ($P < 0.002$). There was a significant ($P < 0.03$) reduction in all the parameters such as total volume (36.6%) and total (36.8%) and free acid (42.6%) as compared to control. RA extract 0.5 g/kg, 1.0 g/kg and cimetidine 200 mg/kg were also tested for their effect on stress induced water restraint gastric lesions; RA extract (0.5 g/kg and 1.0 g/kg) significantly ($P < 0.01$) prevented gastric lesions induced by non-

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steroidal anti-inflammatory agent (indomethacin, 10 mg/kg); the effect was comparable to that of cimetidine 10 mg/kg.

INTRODUCTION

At the Islamic Centre for Medical Sciences (Ministry of Public Health), Kuwait, a herbal formulation (RA) consisting of dried roots of *Withania somnifera* Linn, rhizome of *Alpinia galanga* Wild, corm of *Merendra persica* Linn and roots of *Pyrethrum indicum* DC is successfully being used clinically in the treatment of rheumatoid arthritis. These four plants have been recommended[1-5] for the treatment of rheumatism, sexual debility, diseases of liver and spleen, constipation and as a digestant and carminative. A combination of these plants has been reported[6] to be effective in the treatment of clinical arthropathies. Earlier studies on RA extract indicated significant anti-inflammatory activity using various experimental models[7] and was also devoid of any teratogenic and mutagenic potentials in mice[8]. Recent work[9] showed some functional and behavioural developmental changes in offsprings born to pregnant rats fed with RA extract during pregnancy and lactation period. The roots of *Withania somnifera* has been reported to reduce gastric ulceration and acid production[10]. Gastro-intestinal ulceration is a widely reported toxicological effect of steroidal and non-steroidal anti-inflammatory drugs. The present study was undertaken to investigate the acute and chronic effects of RA extract on gastric mucosa of normal animals. The effect of the extract was also studied in experimentally induced ulcers in laboratory animals.

MATERIALS AND METHODS

Preparation of RA extract:

The four plant materials, namely - the dried root of *Withania somnifera* (50 gm), rhizome of *Alpinia galanga* Wild (25 gm), corm

of *Merendra persica* Linn, (25 gm) and roots of *Pyrethrum indicum* DC (25 gm), were finely powdered and mixed. Every 125 gm of RA powder was extracted with 2.5 litres of Sorensen buffer (KH_2PO_4 and Na_2HPO_4 1/15 M; pH 7.5) at 37°C for 5 hours. During this process, the mixture was stirred continouusly with the help of an electrical stirrer. The supernatant was filtered through Whatman No. 1 filter paper and stored at 4°C and used within one week.

Evaluation of gastrointestinal toxicity in normal rats:

RA extract was orally administered for a period of 1 to 5 months to groups of Wistar Albino rats of either sex (3 months old, 200-250 g) at a dose level of 400 and 800 mg/rat per day along with drinking water (20 and 40 mg/ml). The animals were sacrificed and the stomach was examined for the presence of ulceration and visually scored in arbitrary units of 0-4 according to the severity[11] and compared with the controls. The scoring units were as follows:-

0 = Normal

1 = Scattered haemorrhagic spots and hyperemia.

2 = Deeper haemorrhagic spots and some ulcers.

3 = Haemorrhagic spots and well formed ulcers.

4 = Extensive haemorrhage, ulcers and perforation.

Anti-ulcer studies in Shay rats:

The method developed by Shay et al[12] has been widely used to test anti-ulcer activity. Two groups (13 each) of Wistar albino rats (200-250 g) were fasted for 36 hours but allowed drinking water *ad libitum*. One hour before the pyloric ligation, group I animals were treated with vehicle and served as control.

Group II animals were given RA extract (0.5 g/kg) orally by gavage. The animals were anaesthelised with ether. Under aseptic conditions, a midline incision (1 cm) was made below the Xiphoid process and extended downwards. After cutting through the muscle layer, through the linea alba, the stomach was exposed and the pylorus

was ligated with a cotton thread. The cut ends of the muscle layer and skin was then sutured. The animals were sacrificed after 5 hours and the stomach removed. The stomach contents were collected for estimation of total volume and acids. The stomach was opened along the lesser curvature, mounted on a cardboard and ulcers were examined and visually scored in arbitrary units of 0-4 as described earlier[11]. The volume of the gastric content was recorded, and the contents were centrifuged to get a clear supernatant fluid for determination of total and free acid[13]. This was done by titrating one ml of the clear supernatant of the gastric secretion against 0.01 N sodium hydroxide solution, using methyl orange until the red colour changed into yellowish orange and the volume of the alkali added was recorded (free acid). Then 2 drops of phenolphthalein was added and the titration was continued until a definite red tinge reappeared. The total volume of the alkali added was recorded (total acid). If a yellow colour is obtained on adding the methyl orange it indicates that there is no free acid in the specimen. In that case phenolphthalein is to be added and titrated for total acid content.

Studies on stress-induced gastric lesions in mice:

Groups of male albino mice (30-40 g) were deprived of food for 24 hours and allowed only drinking water *ad libitum*. After the fasting period, animals were divided into 5 groups of 8 to 12 animals each. Groups I and II were treated with Sorensen buffer, 1ml/100 gm orally by gavage whereas groups III and IV were administered RA extract 0.5 and 1.0gm/kg orally respectively. Group V was injected cimetidine 200 mg/kg (Tagamet, SK&F) intraperitoneally. Groups II to V were immobilized in modified stress cages and then immersed to the level of Xiphoid process in water bath with a constant temperature of 37°C for 4 hours[14]. Thereafter all animals were sacrificed. The stomach was excised and examined for the severity of intraluminal bleeding, mucosal

damage, shedding of epithelium and discrete ulcers and visually scored (0 to 4).

Indomethacin-induced gastric ulcers:

Indomethacin (Sigma, USA) suspended in 70% propylene glycol in water (1 mg/ml) was administered orally at a dose of 10 mg/kg at 18 and 36 hr after deprivation of food to four groups of female albino rats (200-250 g). Group I, II and III were given orally buffer 1 ml/100 gm, RA extract 0.5 and 1.0 g/kg respectively and group IV was given cimetidine 10 mg/kg (i.p.) at 17 and 35 hours after deprivation of food. (i.e. one hour before indomethacin administration). Three hours after the administration of the second dose of indomethacin all groups of animals were sacrificed, stomach removed and examined for lesions in the corpus of the stomach and visually scored (0 to 4).

Statistical analysis:

The results were subjected to statistical evaluation by using Student's 't' test or chi-square test wherever appropriate.

RESULTS

Evaluation of gastro-intestinal toxicity of RA extract in normal rats:

Acute and chronic administration of RA extract did not produce any notable signs of gastro-intestinal toxicity. It did not exhibit any significant abnormality both in the cardiac and pyloric portion of the stoamch such as hyperemia, haemorrhage, gastric mucosal lesions, ulcers etc. There was no change in the structural morphology in the treated groups of either sex compared to control.

Anti-ulcer studies in Shay rats:

The results are presented in Table I. Microscopic examination of the incised stomach of RA extract treated animals (group II)

indicated protection against the ulcers induced by pyloric ligation and revealed only scattered areas of hyperemia characteristic of stage I. The mean ulcer score of group I (control) animals was 2.19 ± 0.28 which was reduced significanlty ($P < 0.002$) to 1 ± 0.24 in group II (treated) animals. In the group I (control) animals the mean secretory volume was 11.29 ± 1.13 ml and this was significantly ($P < 0.03$) reduced to 7.15 ± 1.03 ml (by 36.6%) in the group II (treated) animals. The free and total acid contents of the gastric secretion of the control group were 17.37 ± 2.46 and 22 ± 2.85 mEq/lit respectively which were reduced significantly ($P < 0.03$) to 10.7 ± 1.42 and 13.89 ± 1.83 mEq/lit (38.6 and 42.6%) respectively in the treated group.

Effect on stress-induced gastric lesions in mice:

The results are shown in Table II. The mean ulcer score of the group I animals (unstressed and treated with vehicle) was 0.2 ± 0.02 which was markedly and significantly ($P < 0.001$) increased to 2.3 ± 0.34 in group II animals (stressed and vehicle treated). The mean ulcer score of group III (RA extract, 0.5 g/kg treated) animals was significantly ($P < 0.001$) reduced to 0.1 ± 0.1 when compared to that of group II animals. The mean ulcer score of group V (cimetidine treated) animals was also significantly ($P < 0.001$) reduced to 0.25 ± 0.13 compared to that of group II animals. However, group IV (RA extract 1.0 g/kg treated) animals exhibited lesser tendency of protection 1.25 ± 0.32 which was not statistically significant.

Effect on indomethacin induced gastric ulcers:

The results are summarised in Table III. Indomethacin induced a mean ulcer score of 2.1 ± 0.14 in group I animals. When indomethacin treated animals were given RA extract, 0.5 and 1.0 g/kg orally to group II and group III respectively, it was found to reduce the mean ulcer score significantly ($P < 0.01$) to 0.64 ± 0.21 and 0.78 ± 0.24. The mean ulcer score of group IV (cimetidine in combination with

indomethacin) was also significantly ($P < 0.01$) reduced to 0.57 ± 0.20 compared to that of group I (indomethacin alone).

DISCUSSION

The results of earlier studies[7] indicated that RA extract has significant anti-inflammatory activity in various experimental inflammatory models. The formula is used clinically in the treatment of rheumatoid arthritis at the Islamic Centre for Medical Sciences, Kuwait. One of the most common reasons for rejecting a compound from further consideration, even though it has no major life threatening side effects, is a high incidence of gastrointestinal disturbances[15]. Gastric toxicity of non-steroidal anti-inflammatory agents, in the form of increased acidity and ulcerogenicity is well documented[16]. There seems to exist a relationship between anti-inflammatory potency and ulcerogenic activity of anti-rheumatic drugs[17]. The results of the present studies reveal that RA extract did not induce ulcerogenicity on prolonged oral administration to normal rats. The extract did not produce any abnormality in the gastrointestinal tract and there was no change in the structural morphology in the treated group.

Withania somnifera, a major component of RA extract has been reported to reduce gastric ulceration and acid formation[10]. Therefore, the effect of RA extract on experimentally induced ulcers using Shay rats, stress induced gastric lesions in mice and indomethacin induced ulcers in rats was studied. These models involve various mechanisms in the pathogenesis of ulceration or gastric mucosal damage.

The model developed by Shay et al[12] has been used extensively by several workers to test anti-ulcer activity. The rat stomach secretes gastric juice continuously at a constant rate. If a 2-6 hour ligation period is used, the effect of a test compound on the volume of gastric juice, acid and pepsin concentration and output can be measured[18]. RA extract exhibited protection against the ulcers induced by pyloric

ligation and reduced the ulcer score, total volume and gastric acidity compared to controls in Albino rats (Table I).

Hypothermic restraint stress produces disturbances of gastric mucosal microcirculation[19], alteration in gastric secretion[20] and abnormal motility[21]. The RA extract (0.5 g/kg) induced a protective effect on gastric lesions in Albino mice which was comparable to that of cimetidine (Table II). The high dose (1.0 g/kg) treated animals exhibited lesser tendency of protection against restraint-induced lesions compared to the optimal dose (0.5 g/kg). It has been reported that the major component of RA extract namely, *Withania somnifera*, has shown lesser biological activity at higher doses[10]. It has also been observed that very high doses of certain herbal preparations did not show the desired effect as exhibited by the optimal dose[35].

It has been reported that indomethacin causes gastric ulceration[22] and a dose-dependent increase of pentagastrin stimulated acid secretion[23]. In the present investigation it was found that RA extract was able to prevent the gastric damage caused by indomethacin (Table III). Hence, the possibility of inhibition of pentagastrin by RA extract cannot be ruled out.

It has also been postulated that histamine might play an important role in mediating the gastric secretion stimulated by gastrin, vagal excitation and cholinergic actions in both pylorous ligated Shay rat techniques and stress induced restraint ulcers[24]. It has been reported that stress induces mast cell degranulation and histamine release in gastric mucosa[25]. A rise in histidine decarboxylase (an enzyme involved in the synthesis of histamine) activity of the stomach was observed and reported[26] in restraint ulcers which lead to the release of histamine from gastric mucosa. The release of histamine during the stress, in turn, produces damage in the vascularity of mucosa and rupture of histamine dilated capillaries resulting in the production of gastric lesions [27,28]. Our earlier studies[7] revealed that the formulation exhibits significant anti-histaminic activity. Preliminary studies in our laboratory have indicated that RA extract is active in inhibiting the

messenteric mast cell degranulation and associated release of histamine induced by compound 48/80. Further, it has been postulated that anti-allergic compounds might prevent the release of histamine by stabilization of the mast cell membranes[29,30]. It has been reported recently[31] that RA extract was able to stabilize sheep red blood corpulses (SRBC) membranes subjected to hypotonic and heat stresses *in vitro*. Hence, it is likely that RA extract might prevent the release of histamine from mast cells by stabilizing their membrane system. Further, *Withania somnifera* was also found to stabilize the lysosomal membrane of the rat liver both *in vitro* and *in vivo*[10]. Hence, the protective action of RA extract against ulcer formation may also be due to its capacity for the stabilization of lysosomal membrane system.

There are reports on the effect of aspirin-like drugs on platelet aggregation[32,33] and inhibition of prostaglandin synthesis in gastric cells[34], leading to accumulation of precursors of prostaglandins, arachidonic acid, which is diverted for hydroperoxide synthesis[35]. The role of inhibition of prostaglandin synthesis by non-steroidal anti-inflammatory agents has been considered in gastric damage[36]. This is one of the mechanisms postulated for the gastric ulceration caused by the aspirin-like drugs. It is interesting to note that RA extract was found to inhibit the stable phase of carrageenan induced edema (prostaglandin phase) and also to inhibit the edema induced by local injection of PGE_2 in the rat paw[7]. This demonstrates that RA extract is able to inhibit prostaglandin formation. This might be a paradoxical situation. It has been reported that some of the flavonoidal glycosides inhibit both cyclooxygenase[37] and lipoxygenase[38]. The flavonoids are found to reduce gastric mucosal damage induced by non-steroidal anti-inflammatory agents like aspirin, phenylbutazone, indomethacin and ibuprofen[39]. It was reported that a major component of RA extract *Withania somnifera* contains bioflavanoids[40]. *Withania somnifera* is reported to be an anti-inflammatory drug which did not exhibit gastric ulcers on prolonged administration[10]. The preliminary studies (Unpublished data) showed the presence of flavonoidal glycosides in *Merendra persica* (another constituent of RA extract) and in RA extract also. It may be possible

that flavonoidal glycosides in RA extract may be able to inhibit both lipoxygenase and cyclooxygenase leading to the beneficial effects of RA extract.

During the course of the experiments, it has been observed that the gastric content in the RA extract treated group in all the animal models were thick and viscid suggestive of increased mucin production which may act as a protective barrier against ulceration and studies on this aspects are to be initiated. It is suggested that the anti-histaminic, anti-secretory, antacid like activity and a protective effect on the gastric mucosa may be responsible for the anti-ulcer action of the formulation.

It is quite interesting to find an anti-inflammatory formula, RA extract, possessing anti-ulcerogenic activity. Similar actions have been reported for several other anti-ulcer drugs of plant origin such as Carbonoxelene sodium[41] synthesised from liquorice roots, xanthones of *Calophylum inophyllum* and *Mesua fera*[42], nimbidin, a principle isolated from *Azadhirachta indica*[43], a flavonoidal glycoside Gossypin[39] and certain flavonoidal glycoside isolated from *Clerodendron inerme*[35]. The studies on the effects of the individual plant components of the RA extract on experimentally induced ulcers is under progress.

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TABLE I: EFFECT OF RA EXTRACT ON SHAY RATS

Group	Treatment	Secretory Volume (ml)[a]	Acidity in mEq/L[a]		Ulcer Score[b]
			Free Acid	Total Acid	
		mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.
I	Control (n = 13)	11.29 ± 1.13	17.37 ± 2.46	22.00 ± 2.85	2.19 ± 0.28
II	RA extract 0.5 g/kg (n = 13)	7.15 ± 1.03	10.7 ± 1.42	13.89 ± 1.83	1.0 ± 0.24**
	% inhibition	36.6*	42.6*	36.8*	45.6**

a. Results are compared using Students 't' test

b. Results are compared using Chi-square test

* P < 0.03; **P < 0.002

TABLE II: EFFECT OF RA EXTRACT ON STRESS INDUCED ULCER IN MICE

Group	Treatment	Dose g/kg	No. of Animals	Ulcer Score Mean ± S.E.M.	P. Value
I	Unstressed control	Vehicle	12	0.2 ± 0.02	-
II	Stressed control	Vehicle	10	2.3 ± 0.34	< 0.001[a]
III	RA extract	0.5	10	0.1 ± 0.10	< 0.001[b]
IV	RA extract	1.0	8	1.25 ± 0.32	N.S.[b]
V	Cimetidine	0.2	8	0.25 ± 0.13	< 0.01[b]

The results are compared using Chi-square test

a: results compared with that of unstressed control

b: results compared with that of stressed control

TABLE III: EFFECT OF RA EXTRACT ON INDOMETHACIN (10 mg/kg) INDUCED ULCER IN RATS

Group	Treatment	Dose g/kg	No. of Animals	Ulcer Score Mean ± S.E.M.	P. Value
I	Indomethacin		10	2.1 ± 0.14	-
II	Indomethacin + RA Extract	0.5	7	0.64 ± 0.21	< 0.01
III	Indomethacin + RA Extract	1.0	7	0.78 ± 0.24	< 0.01
IV	Indomethacin + Cimetidine	0.01	7	0.57 ± 0.20	< 0.01

1. The results were compared with indomethacin treated group using Chi-square test.
2. RA extract 0.5 and 1.0 g/kg was given orally and cimetidine intraperitoneally twice, one hour before the oral administration of indomethacin at 18 and 36 hr after deprivation of food.

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EVALUATION OF THE REPRODUCTIVE TOXICITY OF CERTAIN HERBAL FORMULATION IN MICE

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Abstract

Recently, a herbal formulation, consisting of the roots of *Withania somnifera* Linn., *Pyrethrum indicum* D.C., corm of *Merendra persica* Linn. and rhizome of *Alpinia galanga* Wild. (2:1:1:1) has been found, in our laboratories, to exert promising anti-inflammatory activity in animal models.

To assess the teratogenic and mutagenic effects of the formulation, male and female mice were given the aqueous herbal extract, mixed with drinking water (12.5 or 25 mg/ml), for 2 hr (9-11 a.m.) daily for 6 days before, 5 days during mating and until day 19 of gestation. Thereafter, the females were sacrificed and fetuses examined. The high dose group had significantly lesser degree of late resorptions and male/female ratio. No difference in gross malformation rate was observed compared with matched controls. The fetuses of the low dose group showed retarded caudal ossification, and those of the high dose group had higher frequency of extra ribs. On the other hand the males had extended treatment for upto 50 days and remated, 5 days later, with untreated females (1:3); all females were sacrificed 10 days after mating period and examined for dominant lethal mutations. There was no difference between the groups in the number of males mated or preimplantation embryonic loss. Post-implantation loss due to mating with low dose males was significantly less. Further, the spermatozoa collected from the cauda epididymis of sacrificed males were examined

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for sperm head abnormalities. There was no significant difference between the groups which may rule out a mutagenic effect of the extract during sperm maturation.

The extract (1g/kg, orally for 5 days) exerted a weak, but significant estrogenic and androgenic activity in overiectomized and castrated adult mice respectively, but not in immature 24-day old mice. The extract did not possess any progestogenic activity.

INTRODUCTION

The medicinal plants *Withania somnifera, Pyrethrum indicum, Merendra persica*, and *Alpinia galanga*, enjoy considerable reputation and have been recommended and used for a variety of diseases in traditional medicine[1-5]. They have been used as anti-rheumatic, anti-inflammatory, aphrodisiac and as a general tonic. *Withania somnifera* is especially used as uterine tonic in leucorrhoea, habitual miscarriage and also in spermatorrhoea and premature ejaculation[6-8]. The combination of these plants has been used effectively in the treatment of clinical arthropathies[9]. The anti-inflammatory activity was evaluated and confirmed experimentally[10], and their combination was pharmaceutically formulated as tablets and being used clinically for the treatment of rheumatoid arthritis in Islamic Centre for Medical Sciences, Ministry of Public Health, Kuwait.

The present study was designed to investigate their possible teratogenic and mutagenic effects in mice, as a part of assessment of their safety.

MATERIALS AND METHODS

Composition of the formulation:

The formulation (R.A.) consists of the powder of dried roots of *Withania somnifera*, Linn. (Solanaceae), rhizome of *Alpinia galanga*, Wild. (Zingeberaceae), corm of *Merendra persica*, Linn. (Liliaceae),

and roots of *Pyrethrum indicum*, D.C. (Asteraceae) in a ratio of 2:1:1:1 parts respectively.

Preparation of the extract:

The extract was prepared by mixing 125 g of powdered RA with 2.5 litre phosphate buffer pH 7.5 (KH_2PO_4/Na_2HPO_4, 1/15 M). The mixture was stirred (250 r.p.m.) using an electrical stirrer (Laboratory Supply Company, West Germany) in a water bath at 37°C for 5 hours. The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate stored in the deep freeze at -20°C, and used within a week. One ml of the filtrate is equivalent to 50 mg of RA extract.

Animals:

Male (35-45 g) and female (25-40 g) Swiss albino mice were used in the present experiment. The animals were maintained on standard pellet diet (86/A; Bugbrooke Mills, Northampton) and water *ad libitum*, unless otherwise stated.

Doses:

In the present experiment, low (L) and high (H) dose levels of RA were given mixed with drinking water or by gavage. These doses were chosen, according to preliminary experiments, to be equivalent to 10 and 20 times, respectively, on mg/kg basis of the maximal allowed clinical doses[9]. The low dose was comparable to the clinical dose based on surface area ratio.

Drug administration:

Three groups of both males and females (10 males and 30 females per group), were selected as control, L and H dose groups. The males were caged individually, while females were caged as 3 animals per cage. Drinking water was removed from all cages at 7-9 a.m. daily. Both males and females were given water, or extract

mixed with drinking water (12.5 or 25 mg/ml) for 2 hours (9-11 a.m.) for the control, L and H dose groups respectively.

Fertility and teratogenicity studies:

Administration of extracts was continued daily for 6 days before, and during a mating period of 5 days. The extract consumption was measured daily. Mating of animals was performed by caging 3 females with the same male overnight for 5 consecutive nights or until the animals conceived. In the morning, the females were examined for the presence of vaginal plugs. The day of the presence of a vaginal plug was designated day one of pregnancy. The administration was continued until day 19 of gestation, when the animals were sacrificed by cervical dislocation, and the fetuses removed by Caesarian section. The number of live and dead fetuses and resorption sites were counted and the fetuses removed, weighed and examined macroscopically for gross defects. They were then fixed in 70% alcohol and subsequently dissected to examine for visceral defects. All fetuses were then alizarin stained and the skeletons examined. All uteri with no signs of pregnancy were stained with ammonium sulphide to examine for the presence of implantation sites. In addition, the number of males that mated and of females that became pregnant in each group were also recorded to assess the effect on fertility.

Mutagenicity studies:

A. Dominant lethal mutations:

Males, from the previous experiment, were continuously given the extract for up to 50 days. They were remated, 5 days later, with untreated females, by caging each male with 3 females for 5 days[11]. All females were sacrificed by cervical dislocation 10 days after the end of the mating period. The uterine horns were examined for live and dead fetuses and resorption sites. In addition, the number of

males that mated and of females that became pregnant in each group were also recorded.

B. Sperm head abnormalities:

The same males were then sacrificed, within a week, and the two cauda epididymes were excised. Each was placed in one ml saline, finely minced with scissors and left for one minute for the spermatozoa to diffuse out. A drop of the suspension was spread on a slide, air dried, fixed in absolute alcohol, and stained for 15 min in a 1% aqueous Eosin Y solution. Two slides per mouse were screened blindly by 3 different investigators for sperm head abnormalities[12]. Results are expressed as mean percentage sperms with abnormal heads per group of animals.

Endocrinological studies:

1. Estrogenic activity:

The estrogneic activity of the extract was assessed by measuring uterine weight of overiectomized and immature female mice as well as estrus induction in adult mice[13].

i. Overiectomized adult mice:

Four groups of 5,5,7 and 11 adult female mice, were over-ictomized under nembutal anaesthesia (40 mg/kg, i.p. of nembutal sodium, Abbott Laboratories). Five days later, the animals were given phosphate buffer (20 ml/kg, by gavage), estradiol (0.1 μg/ mouse, s.c.) and extract (500 and 1000 mg/kg, by gavage) respectively, once daily for 5 days. The animals were killed by cervical dislocation, 24 hours after the last administration. The uterine horns were excised, uterine contents were gently squeezed out; and the wet weight was determined.

ii. Immature mice:

Three groups of 11,3 and 13, 24-dayold female mice were given phosphate buffer (20 ml/kg, by gavage), estradiol (0.1 μg/mouse, s.c.) and extract (1000 mg/kg, by gavage), respectively, once daily for 5 days. The animals were killed by cervical dislocation, 24 hours after the last administration. The uterine horns were dissected out, their contents were gently squeezed out, and the wet weight was determined. The body weight of the animals was also determined at the beginning and the end of the experiment.

iii. Induction of estrus in adult mice:

Two groups each of 9 adult female mice, were used. Vaginal smears were taken from each animal twice daily for 5 days before and during a treatment period of 5 days. Phosphate buffer (20 ml/kg) and extract (1000 mg/kg) were given by gavage once daily, respectively. The number of animals with positive estrus smear was recorded.

2. Progestogenic activity:

The progestogenic activity of the extract was evaluated by measuring the ability to maintain pregnancy in overiectomized mice[14]. Two groups of 7 and 9 pregnant mice were given water or 25 mg/ml extract in the drinking water respectively from day 8 to 19 of pregnancy. On day 10 of gestation, the animals in both groups, were overiectomized under nembutal anaesthesia (40 mg/kg, i.p.). Four pregnant mice were Sham operated at day 10 of gestation and used as a control non-overiectomzied group. At day 19 of gestation, the animals were killed by cervical dislocation, and the uterus was examined for the presence of live fetuses, resorptions and implantation sites.

3. Androgenic activity:

The androgenic activity of the extract was investiged by weighing prostate and seminal vesicles of castrated and immature male mice[14].

i. Castrated adult mice:

Two groups of 6 and 5 adult male mice were castrated under nembutal anesthesia (40 mg/kg, i.p). Five days later, the extract was given by gavage at a dose of 1000 mg/kg, once daily for 5 days to the first group. The other group was given 20 ml/kg phosphate buffer. Twenty-four hours after the last administration, the mice were sacrificed by cervical dislocation, and the ventral prostate and seminal vesicles were dissected out. The wet weight was determined and compared with that of non-castrated adult males.

ii. Immature mice:

Two groups of 12 and 11, 24-day old male mice were given phosphate buffer (20 ml/kg) and the extract (1000 mg/kg) orally by gavage once daily for 5 days respectively. Twenty-four hours after the last administration, the animals were killed by cervical dislocation, and the ventral prostate and seminal vesicles were dissected out and the wet weight was determined. The body weight of the animals was also determined at the beginning and the end of the experiment.

Statistical methods:

The results were analyzed using Student's 't' test, Chi-square test or Wilcoxon rank sum test, where appropriate.

RESULTS

Body weight gain and extract consumption:

Body weight of male and female mice as well as their consumption of the extract during treatment period is shown in Figure 1. The extract was given in the drinking water for 2 hours

daily in two concentrations (12.5 and 25.0 mg/ml) for both males and females. The animals gradually consumed an increased amount of the extract during the first week of experiment, and reached virtually a constant volume by the end of the second week. The average extract consumed by males was 247 ± 22.9 – 729 ± 101.9 and 600 ± 63.6 - 1777 ± 225.0, and by females was 141 ± 29.8 – 342 ± 35.0 and 303 ± 70.5 – 808 ± 60.3 mg/kg daily for L and H dose groups respectively. The body weight gain of the males and females in both treated groups was similar to that of the control groups during the treatment period. Female mice in all groups progressively increased in body weight towards the end of gestation period with a concomitant decrease in the dose taken as mg/kg. The volume taken daily was, however, not changed.

Fertility and teratogenicity studies:

The results are shown in Table 1. Two males in each of control and L dose groups failed to mate with any of the 3 females caged with them during 5 successive nights. Therefore, 6 females from each of these two groups were excluded, besides another one pregnant female from H group, escaped from the cage and died. From the remaining animals, 2 females from each of control and H group did not mate successfully and another one control animal which showed positive vaginal plug was found non-pregnant. There was no significant difference between the groups in the number of males that mated or of females that became pregnant at the end of 5 days mating period. Furthermore, there was no significant difference in the number of live fetuses or advanced resorptions/litter. Delayed resorption rates were significantly lower in H group. A dose related decrease in the male/female ratio was observed and was significant at the high dose level ($P < 0.02$). The gross external malformations, were two runted fetuses from two litters, one fetus with hydrocephallus and one fetus with subcutaneous haemorrhage, in the control group; four runted fetuses from two litters, one fetus with full length cleft palate and one fetus with subcutaneous haemorrhage in the low dose group; three runted fetuses from

different litters in the high dose group. There was no significant difference in fetal body weight between the groups.

Skeletal examinations:

The results are shown in Table 2. The fetuses in the L group had retarded caudal ossification, and those of H group had higher frequency of extra ribs. There was no difference between the groups in the degree of ossification or frequency of abnormal fusions of sternebrae.

Mutagenicity study:

A. Dominant lethal mutations:

The results are shown in Table 3. The same two males in the low dose group which failed to mate in the teratology experiment, failed to mate again at the end of the treatment period. The two control males which did not mate earlier, however, succeeded to mate with 1 and 3 females respectively. Another control male died during the experiment. There was no significant difference between the groups in the number of males mated or a total number of implants as a measure for (pre-implantation loss). Post-implantation loss (mutagenic index), due to mating with low dose males, however, was significantly less than that of the control group ($P < 0.03$).

B. Sperm head abnormalities:

The results are shown in Table 3. When the males were killed and the sperms were examined for head abnormalities, it was found that, the two males in the low dose group, which failed to mate both at the beginning and end of the experiment, had oligospermia with very high rate of sperms with abnormal heads (63 and 80%) compared with an average of about 4% in the other animals. Their values, therefore, were excluded from the calculation of the mean of the group. In all animals, however, there was no significant

difference between the groups in the rate of sperm abnormalities using the Wilcoxon rank sum test.

Endocrinological studies:

1. Estrogenic activity:

The effect of oral extract administration for 5 days on the uterine weight of overiectomized and immature female mice is shown in Figure 2. Administration of 0.1 μg/mouse estradiol s.c. once daily for 5 days produced a marked increase in the wet uterine weight of both overiectomized (from 31.4 ± 3.2 to 251 ± 24.0) and immature (from 17.0 ± 1.0 to 54.4 ± 6.7 mg) mice. In overiectomized adult mice, the extract produced an increase of 22 and 90% in uterine wet weight, when given in doses of 500 and 1000 mg/kg orally respectively. The effect of the high dose was significant ($P < 0.02$). In immature 24-day-old female mice, however, 1000 mg/kg extract had no effect on uterine or body weight.

The effect of oral extract administration on estrus induction in adult mice is shown in Figure 3. Figure 3a shows results extracted from Table 1, where the cumulative % of pregnant mice were recorded during the 5 days mating period. In the third day of mating, 72.7, 83.3 and 92.5% of the control, low and high dose levels of the extract respectively, became pregnant. The results, though dose related are not significantly different, and the effect of the higher dose reached a probability of 0.062. Figure 3b shows per cent of adult non-pregnant mice with positive estrus in a vaginal smear, 5 days before and during treatment with 1000 mg/kg extract orally. Treatment caused a significant increase ($P < 0.03$) of animals with positive estrus during treatment period than before treatment. Administration of phosphate buffer, however, did not produce any significant change.

2. Progestogenic activity:

In all animals, live implants were seen in both utrine horns during overiectomy at day 10 of gestation. Sham operated group (4

animals), maintained pregnancy and had a total of 39 live fetuses (range 8-11) and one resorption in each litter at day 19 of gestation. In overiectomized non-treated group (7 animals), no live fetuses were found, 2 animals had 8 and 9 resorptions and 5 animals had 52 implantations sites (range 8-13). In overiectomized extract treated group (9 animals), no live fetuses were found, all animals had 71 resorptions (range 1-12), and 7 animals had also 28 implantation sites (range 3-11).

3. Androgenic activity:

The effect of 1000 mg/kg RA extract orally for 5 days on prostate and seminal vesicles weight of castrated and immature male mice is shown in Figure 4.

In castrated adult male mice, administration of 1000 mg/kg extract for 5 days caused an increase in the weight of prostate and seminal vesicle by 31.3% (from 65.1 ± 3.3 to 85.5 ± 6.3 mg, $P < 0.02$). Non-castrated adult male mice had prostate and seminal vesicle weight of 185.6 ± 6.3 mg (4 animals).

In immature 24-day-old mice, however, there was no significant change in the weight of prostate and seminal vesicle (from 22.6 ± 3.2 to 26.7 ± 3.2 mg). There was also no effect on body weight gain of these animals.

DISCUSSION

The maximal dose of the herbal preparation (RA) recommended for treatment of clinical arthropathies is 4 g/patient daily[9], which is about 57 mg/kg. This dose is equivalent to 520 mg/kg for mice based on surface area ratio, i.e. about 10 times the clinical dose as mg/kg. In the present experiment, the extract was given to mice mixed with drinking water (in two concentrations, 12.5 and 25 mg/ml), for 2 hr, daily during the whole treatment period. The maximal extract consumed by males was 929 and 1777 mg/kg, and by females was 342 and 808 mg/kg for low (L) and high (H) dose groups respectively. Therefore, male mice were exposed to maximal concentrations of the extract of 13 and 31 and and females to 6 and

14 times that of the human dose (mg/kg) for L and H groups respectively.

The extract in the doses given did not show any signs of toxicity on male and female mice nor affected their body weight gain during treatment peirod.

Fertility and teratogenicity studies:

Administration of the extract to males and females started 6 days before mating. This covered one estrus cycle in the females and will also detect effects on mature sperms due to exposure of the males to the extract. Further, this design may also detect changes in sexual behaviour, such as changes in libido or potency. These changes are most likely to occur with agents that interfere with hormone secretion or have a central or a peripheral action on the nervous system[15]. This would be reflected as changes in the number of both males and females that successfully mated. In the present experiment, there was no significant difference between the groups in the number of males mated or of females that became pregnant at the end of 5 days mating period. On the third day of mating, however, there was a dose-related higher number of females that became pregnant being 72.7, 83.3, and 92.5% for control, L and H groups respectively. These slight differences may suggest some hormonal changes in the animals as a result of extract exposure, particularly its major component, *Withania somnifera. Withania somnifera* has been reported to contain the steroidal lactones, Withanolides[16], and has a reputation in traditional medicine in the treatment of reproductive disorders[6-8]. The extract produced a dose-related reduction in delayed resorptions and male/female ratio. The effect of the high dose was statistically significant ($P < 0.02$). Similarly a decrease in male/female ratio was found in the offspring born to rats given the extract 15 days before mating and through gestation period[17]. The results of both experiments (in mice and rats) may indicate that the change in offspring sex-ratio is a result of effects on the females. Males in the rat experiment were not treated. The change in sex-proportion could be due to variation in

vaginal and intracervical pH as a result of direct actions of the extract or through a hormone-mediated effect. Changes in pH level affect the motility of spermatozoa containing X and Y chromosomes[18]. In man, male zygotes are formed earlier in the cycle than female zygotes[18,19], and this is related to maternal hormone levels[20]. The finding in the present experiment that the extract induced estrus in mature female mice, could mean that at the time of mating, the treated animals were already late in the cycle which favours fertilization of female zygotes.

There was no significant difference between the groups in fetal weight or frequency of gross malformations. Delayed caudal ossification in the L group and high frequency of extra ribs in the H group, were observed. The delayed ossification was not dose-related, and could reflect the slight, non-significant, decrease in fetal weight in the same group. Therefore, it is not normally regarded as specific embryotoxic effect[15]. The absence of other congenital abnormalities and anomalies in the treated groups, may regard the finding of extra ribs of no toxicological significance[15].

Mutagenicity studies:

Mutagenicity tests are now widely accepted by regulatory agencies as being of some value for prediction of carcinogenic potential. Damage to DNA in germ cells may lead to genetically altered gametes, resulting in early abortions, fetal deaths, stillbirths, or abnormally developed offspring[21]. Chemicals causing chromosomal damage (breaks, re-arrangement, single gene mutation, etc.) are more likely to affect males than females when exposure occures during adulthood. In males, the production of sperm in adult life is continuous and repeated cell divisions during the sperm cycle maximize the opportunities for chemical attack on the chromosomes[15]. The whole process of spermatogenesis takes from about 8 weeks in mice to 10 weeks in rats and man[15]. Chemicals can affect any one or several stages of spermatogenesis and the effect observed depends on the stages affected.

The dominant lethal mutation test in male mice can demonstrate the response of different stages of development of male germ cells to the suspected drug. A dominant lethal mutation is a genetic change that results in the death of the conceptus that inherit it[22]. The main type of genetic damage it detects is chromosomal breakage with a consequent increase in non-viable embryos[22,23]. The test can also detect changes in male fertility. Administration of the extract, in the present experiment, covered the whole period of spermatogenesis in mice, and the results, showed no effect of the extract on fertility of the males or on pre-implantation embryonic loss. Post - implantation embryonic loss (mutagenic index), due to mating with L dose males, however, was significantly low.

The sperm-head abnormality assay is an *in-vivo* technique for the identification of agents capable of causing an increase in the incidence of sperms with morphologically abnormal head shapes in mice[12]. Insults to the spermatogonia or early spermatocytes can lead to a disruption of normal differentiation of the morphology of sperm cells. The shape of sperms is polygenically determined and has a high heritability[12]. About 90% of the agents which induce sperm abnormalities in mice are carcinogens or mutagens, and the interference with the mechanism controlling sperm shape in exposed males may be associated with the induction of transmissible mutations in mice. The results, of the present experiment, showed no treatment related change in the rate of sperm head abnormalities. It is interesting to find that the two males in the low dose group, which failed to mate both at the beginning (teratology experiment) and end (dominant lethal mutation) of the experiment had oligospermia with very high rate of sperms with abnormal heads. This finding may indicate that the test can detect changes in fertility of male mice.

Endocrinological studies:

It was found, in the present experiment, that extract administration increased the cumulative pregnancy rate during the mating period, and altered the sex-proportion of their offspring. Both

effects were dose-related and could be hormone-mediated, as discussed previously. Therefore, experiments were designed to investigate that possibility.

It was found that extract administration increased uterine weight of adult (overiectomized), but not of immature female mice. It also induced estrus in adult female mice. These results may indicate that the extract had a slight but significant estrogen-like activity in adult female mice. The effect could be due to direct effect on the uterus or through stimulation of synthesis of gonadal steroids from the adrenals. The adrenals have the biochemical capacity to produce appreciable amounts of gonadal steroids (estrogens, androgens and progestogens)[24]. Liver and fatty tissues can also produce small quantities of estrogens from circulating androgens of adrenal or ovarian origin[25]. The lack of an effect of the extract on uterine weight of immature mice could be related to lower sensitivity of immature uterus to the action of estrogens. In the present experiment an injection of estradiol (0.1 μg/animal, s.c.) produced 7 fold increase in uterine weight of overiectomized adult mice, compared with only 2.2 fold increase in immature mice. The extract produced slightly less than one fold increase in uterine weight of overiectomized adult mice.

The extract did not show any progestogenic activity since it failed to maintain pregnancy in overiectomized mice, in the present experiment, and to prevent estrogen (1 μg/mouse)-induced reduction in the number of embryos implanted, when given to mice during pre-implantation period (unpublished observation), according to the method described by Gidley-Baird et al[26].

On the other hand, administration of the extract increased the weight of prostate and seminal vesicle of adult (castrated) by 31%, but not of immature male mice. It also significantly increased the weight of prostate and seminal vesicle of non-castrated adult mice by 30%, with a non-significant increase (from 0.903-1.112 ng/ml, 23%) of plasma testosterone level measured using radio-immunoassay (unpublished observation). The testosterone level of castrated mice was undetectable, and the estrogen level (measured by radio-

immunoassay) of overiectomized and intact mice was not changed by extract administration (unpublished observation). These findings indicate that the extract may increase the synthesis of gonadal steroids (possibly androgens) in the adrenals of both males and females. In females, body fat is an important site for the aromatization to estrogen of circulating androgens of adrenal and ovarian origin[25]. The suggestion of adrenal involvement may be supported by the preliminary finding, that extract administration for 4 months increased the adrenal weight of male and female rats (unpublished observation). The extract could enhance steroid synthesis through an increase of steroidal precursors. Steroidal lactones (Withanolides) of *Withania somnifera*, could be one factor; or the increase in serum cholesterol level due to extract administration[17], could be another factor. It was shown that adrenal steroids are derived from plasma cholesterol[27]. Further work is in progress to investigate these possibilities.

In conclusion, the extract did not exhibit major signs of reproductive toxicity, teratogenicity and mutagenicity in mice. It exerted a weak, but significant estrogen and androgen-like activities in female and male mice respectively. Experiments are in progress, on other species to further assess its safety for use during pregnancy.

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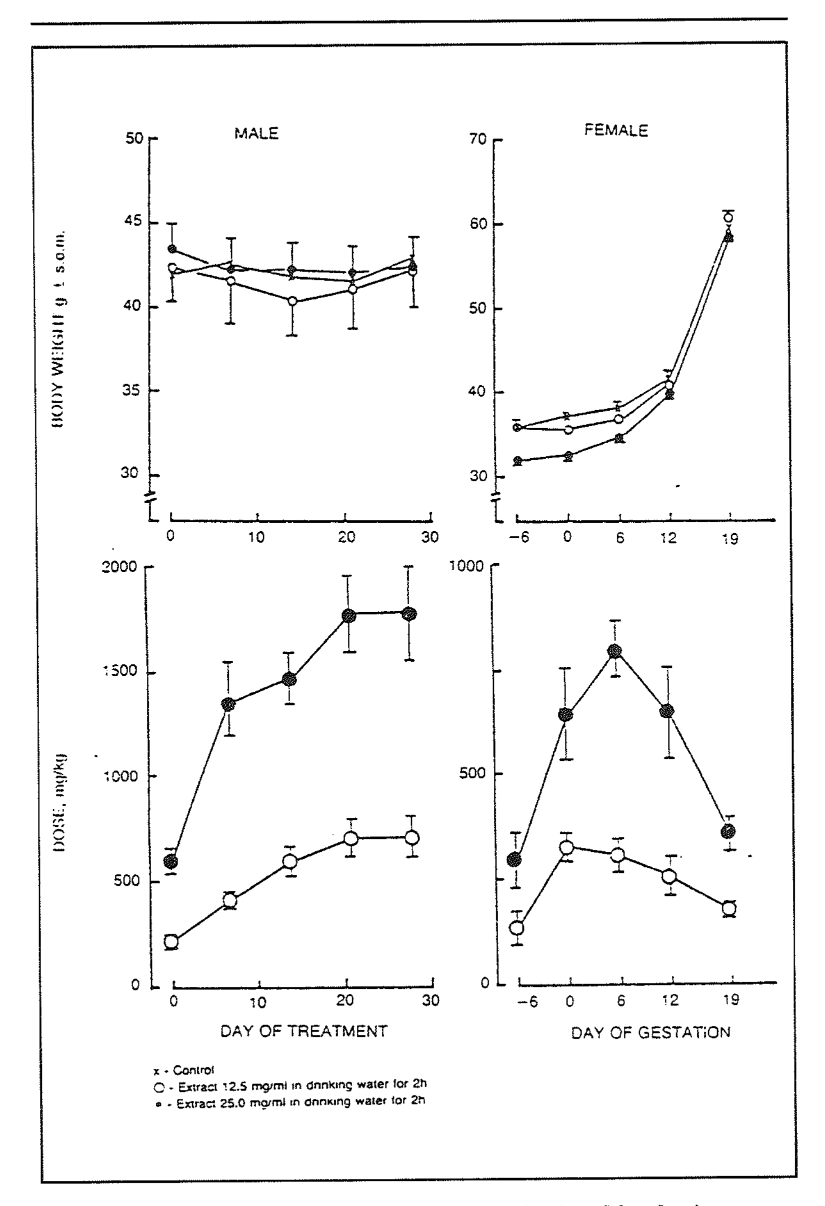


Fig. 1: Body weight and extract consumption of male and female mice during treatment period.

TABLE 1: TERATOGENIC EFFECTS OF THE EXTRACT GIVEN IN THE DRINKING WATER 6 DAYS BEFORE MATING AND THROUGH GESTATION UNTIL DAY 19 OF PREGNANCY

Parameters	Control	Extract, 12.5 mg/ml	Extract, 25 mg/ml
No. of males mated/Total No.	8/10	8/10	10/10
No. of females with plugs/Total No.	22/24	24/24	27/29
Total no. of implants/No. of litters	244/21	294/24	324/27
X ± s.e.m.	11.6 ± 0.47	12.3 ± 0.5	12.0 ± 0.54
Live fetuses/No. of litters	213/21	260/24	299/27
X ± s.e.m.	10.1 ± 0.56	10.8 ± 0.5	11.1 ± 0.63
Advanced resorptions/No. of litters	17/10	22/14	23/11
(%)	(6.97)	(7.48)	(7.1)
Delayed resorptions/No. of litters	14/9	12/11	2/2
(%)	(5.74)	(4.08)	(0.62)*
No. of females (%)	90 (42.1)	128 (49.2)	160 (53.5)*
No. of males (%)	124 (57.9)	132 (50.8)	139 (46.5)*
Dislocated hind limbs (%)	16 (7.5)	23 (8.9)	32 (10.7)
Mean fetal weight g ± s.e.m.	1.49 ± 0.06	1.40 ± 0.04	1.48 ± 0.05
Other malformations	2/2 runt	4/2 runt	3/3 runt
	1 Haem.	1 Haem.	-
	1 Hydrocephallus	1 F.L.C.P.	-

*: $P < 0.02$ compared with the control group using Chi-square test.

TABLE 2: SKELETAL ANOMALIES IN FETUSES EXPOSED TO THE EXTRACT DURING PREGANCY

Treatment	No. examined	Retarded ossification		Skeletal variants	
		Sterebral (%)	Caudal[a] (%)	Extra ribs (%)	Malformed Sternebrae (%)
Control	214	33 (15.4)	60 (28.0)	4 (1.9)	37 (17.3)
RA (Low dose 12.5 mg/kg)	260	51 (19.6)	102 (39.2)**	10 (3.9)	61 (23.5)
RA (High dose 25.0 mg/kg)	299	45 (15.1)	94 (31.4)	16 (5.4)*	67 (22.4)

a: No. of fetuses with less than 8 ossified caudal vertebrae

Results compared with that of the control group using Chi-square test *: $P < 0.05$ **: $P < 0.01$

TABLE 3: EFFECTS OF THE EXTRACT GIVEN IN THE DRINKING WATER FOR 50 DAYS TO MALE MICE, ON DOMINANT LETHAL MUTATIONS (WHEN MATED TO NORMAL FEMALES, 5 DAYS LATER); AND ON SPERM HEAD ABNORMALITIES

Parameters	Control	Extract, 12.5 mg/ml	Extract, 25 mg/ml
Dominant lethal mutations:			
No. of males mated/ Total No.	9/9	8/10	10/10
No. of females pregnant/Total No.	21/27	18/30	22/30
Total No. of Implants	226	216	256
X ± s.e.m.	10.8 ± 0.76	12.0 ± 0.39	11.6 ± 0.44
Live fetuses (%)	200 (80.5)	208 (96.3)	236 (92.2)
X ± s.e.m.	9.5 ± 0.78	11.6 ± 0.43*	10.7 ± 0.49
Resorptions (%)	26 (11.5)	8 (3.7)	20 (7.8)
Sperm Head Abnormalities:			
Sperms with abnormal head mean % ± s.e.m.	4.43 ± 0.29	4.33 ± 0.74	4.9 ± 0.58
(No.)	(9)	(8)	(10)

*:$P < 0.03$ compared with the control group using Student's 't' test.

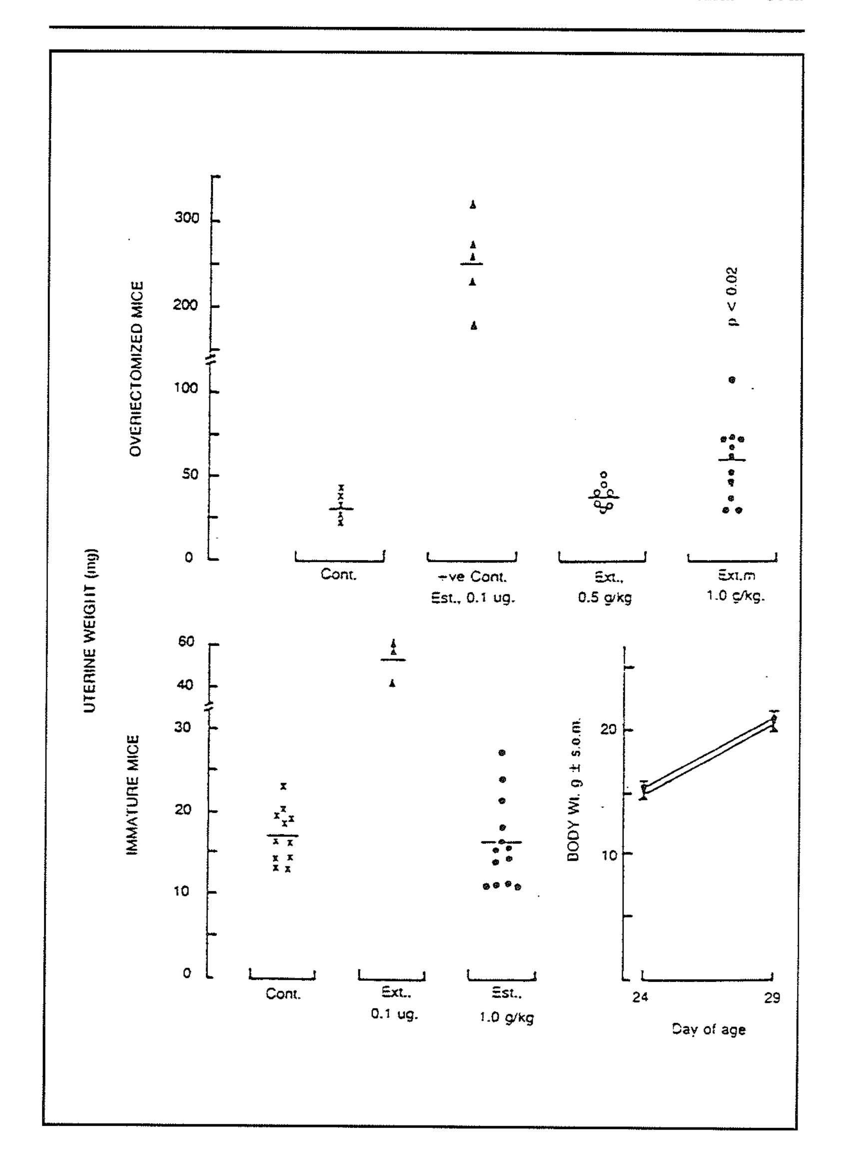


Fig. 2: Effect of extract administration (1 g/kg, orally for 5 days) on uterine weight of mature overiectomized mice, and on uterine and body weight of immature (24 days old) mice.

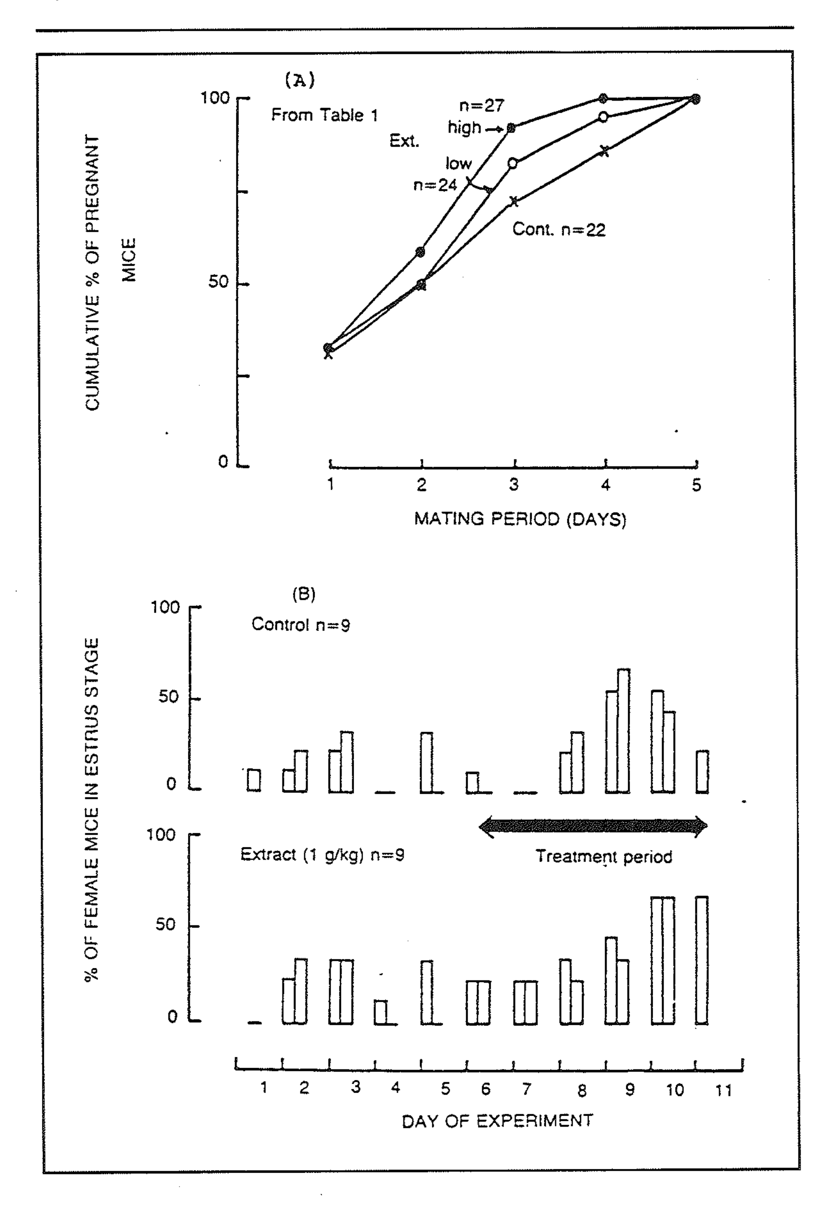


Fig. 3: Effect of extract administration on pregnancy rate (A), and on induction of estrus (B) in mature mice.

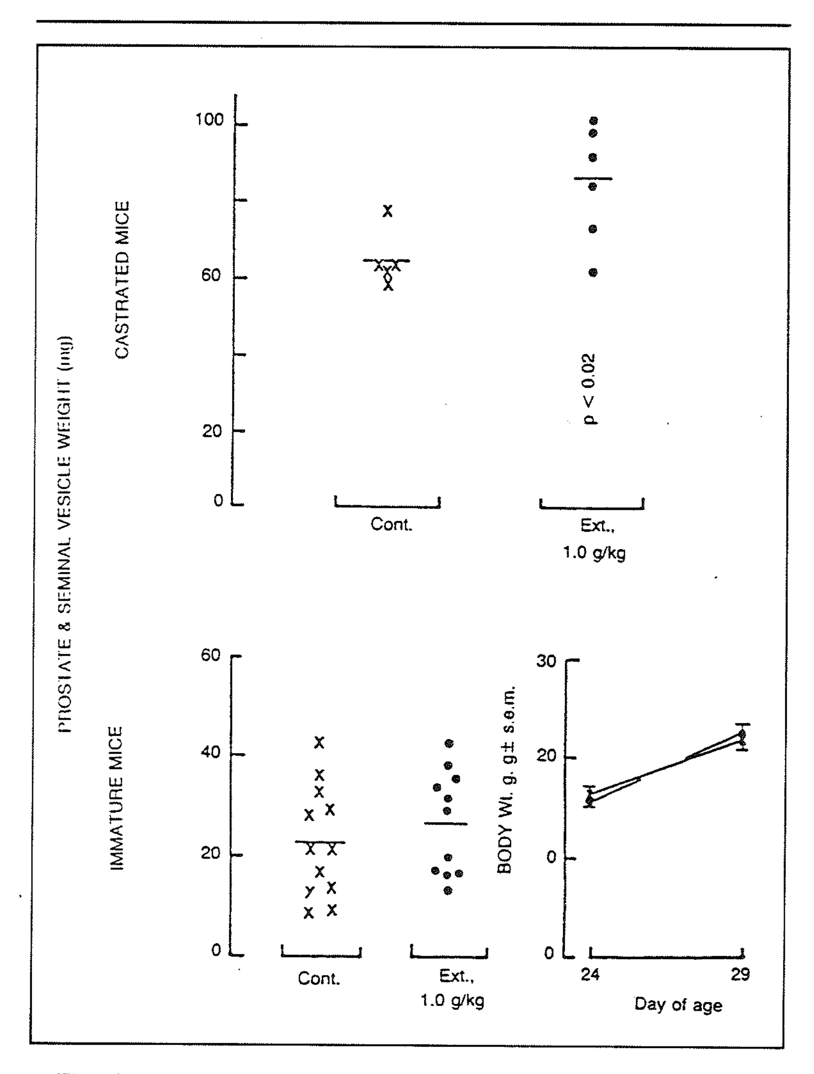


Fig. 4: Effect of extract administration (1 g/kg, orally for 5 days) on the weight of prostate and seminal vesicle of mature (castrated) and immature (24 days old) mice; as well as on body weight of immature mice during the treatment period.

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FUNCTIONAL AND BEHAVIOURAL TERATOLOGICAL STUDIES OF A CERTAIN HERBAL FORMULATION IN RATS

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Abstract:

The herbal formulation consists of the roots of *Withania somnifera* Linn., *Pyrethrum indicum*, D.C., corm of *Merendra persica* Linn. and rhizome of *Alpinia galanga*, and is being used in the treatment of Rheumatoid Arthritis in Islamic Centre for Medical Sciences. Female rats (F_0) were given the aqueous extract of the formula in two dose levels (400 and 800 mg/rat/day) as 20 and 40 mg/ml of drinking water for low (L) and high (H) dose groups respectively, 15 days before placement for mating, through gestation and parturition until day 25 post-partum. The physical and behavioural development of their offspring (F_1) were assessed up to 90 days of age.

Treatment had no effect on body weight gain of F_0 females during pregnancy or length of gestation. There was a dose related increase in the number of live pups and female ratio per litter. The number of stillbirths in the H groups, however, was significantly higher. Body weight gain was less in the treated males and females upto 90 days of age. There was a delay in ear and eye opening of L group. Hair growth of L and H offspring was advanced than the control while there was no difference between the groups in survival, tooth eruption, descent of testicles or vaginal opening.

Assessment of reflex and motor development of F_1 animals revealed a loss of cliff avoidance of H group at day 7 of age, and an increase in grip strength of L group at day 13 of age respectively, in

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swimming performance at 5-14 days of age and in ability to stay on a rotating rod at 30-37 days of age.

There was no difference in an auditory startle response at 14 days of age or jumping down test at 10 days of age. At day 21 of age, however, more of H group jumped down from the high height than the controls.

Assessment of behaviour of F_1 offspring, 40-90 days of age, showed that treated males were hypoactive during the first trial in the activity cage. There was no difference in exploration or repetitive behaviour of treated females on a hole board, while H group had higher emotionality during the first trial. A significant dose related increase (females) and decrease (males) in the number of conditioned avoidances was observed. Male rats in both treated groups made more errors until learned a swimming maze.

When F_1 male rats were sacrificed at 120 days of age, a dose related increase in serum cholestrol and decrease in serum GPT levels was observed. An increase in serum glucose (18%) and blood urea nitrogen (14%) was also observed in H groups.

Fertility index of F_1 rats was 81.3, 59.1 and 95.0% for control, L and H groups respectively. The number of F_2 pups born alive to the treated groups was significantly higher, and 72-93% of all pups survived up to day 21 of age.

Based on the functional alterations observed in the present study, it is advisable not to give these herbal agents to women during pregnancy or lactation period.

INTRODUCTION

From time immemorial, man has used the extraordinary medicinal properties of certain herbs either singly or in combination to treat several baffling diseases. The medicinal plants, *Withania somnifera, Pyrethrum indicum, Merendra persica* and *Alpinia galanga* have been advocated in the treatment of several diseases[1-5],

such as rheumatism, sexual debility, liver and spleenic disorders. *Withania somnifera*, has been recommended as an uterine tonic in leucorrhoea and in the treatment of spermatorrhoea, premature ejaculation, constipation, chronic jaundice and fever[6-8]. The combination of these plants has been reported to be effective in the treatment of clinical arthropathies[9] and is being pharmceutically formulated as tablets (RA) and used clinically in the Islamic Centre for Medical Sciences, Kuwait, for the treatment of rheumatoid arthritis. Earlier studies in our labaratory have indicated the anti-inflammatory[10] and anti-ulcer[11] activity of the formulation experimentally. Further, it was also reported that the RA extract did not exhibit teratogenic and mutagenic potentials in mice[12].

The requirement to test animals for postnatal effects of human medicines was introduced recently in several countries. In the U.K. the requirement was introduced in 1975 with general guidelines which state that, in the fertility and perinatal studies, in addition to testing the reproductive capacity of the offspring, the late effects of the drug on the progeny, in terms of auditory, visual and behavioural function should be assessed. Since, the RA preparation is the most commonly prescribed formula in our Centre, and an increasing number of patients are using it, including women during child bearing age, it was felt necessary to investigate its effect on postnatal physical, functional and behavioural development in the rat, in the course of assessment of their safety.

MATERIALS AND METHODS

The herbal formulation (RA) consists of the roots of *Withania somnifera* Linn. (Solanaceae), *Pyrethrum indicum*, D.C. (Asteraceae), corm of *Merendra persica*, Linn. (Liliaceae), and rhizome of *Alpinia galanga*, Wild. (Zingeberaceae) in ratio of 2:1:1:1 parts respectively.

Female albino rats (parental, F_0) weighing 230-260g, were given the aqueous herbal extract, prepared as described previously[12], in two dose levels (400 and 800 mg/rat/day) as 20 and 40 mg/ml of drinking water for animals for low (L) and high (H) dose groups respectively. The control group was given tap water. The extract was given to animals daily for two hours, a time which was sufficient for the animals to drink what was given to them. Treatment started 15 days before placement for mating and continuously, through gestation and parturition until day 25 post-partum. Mating was performed by placement of 3 females with one untreated male for 2 h (8-10 a.m.) for 5 consecutive days, and successful mating was confirmed by the presence of spermatozoa in the vaginal smear. Pregnant animals were caged individually, and within 24 h after parturition, the offspring (F_1) were culled to 8 per litter and their physical and behavioural development were assessed up to 90 days of age. The reproductive performance of F_1 mature rats was also tested. The physical development of their offspring (F_2) was observed up to 21 days of age.

Physical development:

This was assessed by measuring or observation of the following parameters: survival and body weight at different ages, ear opening at 2-3 days of age, hair growth at 3-5 days of age, tooth eruption at 9-12 days of age, eye opening at 14-16 days of age, descent of testicles at 27 days of age and vaginal opening at 30-42 days of age.

Reflex and motor development:

Surface righting reflex was assessed at 4-6 days of age, cliff avoidence at 7-9 days of age, swimming performance at 5-14 days of age, grip strength at day 13 of age, righting in mid-air at 16-21 days of age, and ability to stay on a rotating rod[13] at 30-33 days of age.

Sensory development:

Development of functional hearing was assessed by measuring an auditory startle response at day 14 of age. Olfaction and visual development were assessed in a jumping down test at 10 and 21 days age respectively[14].

Behaviour:

Spontaneous activity, emotionality and habituation of F_1 offspring were measured using an activity cage (Ugo Basile, Italy) at 39-50 days of age, exploration and repetitive activity were assessed using swimming E-shape maze and automatic reflex conditioner (Ugo Basile, Italy) at 70-90 days of age. Most of these tests have been reported in details[16-18].

Fertility and reproductive function of F_1 rats:

At 90-120 days of age, males and females in each group were mated by placing one male with one female (sibling matings are avoided) for 5 successive days, and then separated and returned to single cages. Females that had not littered after the maximum time allowed for littering subsequent to mating are judged not to be pregnant and were remated for another 5 days. Pregnant females were allowed to deliver and nurse their young. The physical development of the offspring (F_2) was observed up to day 21 of age as shown for F_1 rats.

Biochemical funtions :

At 120 days of age, F_1 males were sacrificed after an overnight food deprivations. Blood was collected from the retro-orbital sinus under Nembutal anaesthesis (40 mg/kg. i.p). The blood was allowed to clot, and serum was separated by centrifugation and used for the determination of glucose, blood urea nitrogen (BUN), albumin, total proteins, total bilirubin, cholesterol, triglycerides, creatinine, alkaline phosphatase, glutamic oxalacetic transminase (GOT), and

glutamic pyruvic tansaminase (GPT), using Automatic Clinical Analyser (ACA, SX, Dupont).

Statistical methods:

The results obtained for males and females separately and in combination for each group were analyzed using Student's 't' test or Chi-square test where appropriate.

RESULTS

The effects of RA on fertility, gestation and litter size are shown in Table I. Out of 20 females in each group, at the start of the experiment, only 7,7 and 6 animals were pregnant at term in the control, L and H groups respectively. Treatment had no effect on length of gestation period, or body weight gain of parental animals during pregnancy. Control rats increased in weight from 273 ± 8.5g to 365 ± 13.2g, L group from 242 ± 4.5g to 332 ± 7.6g, and H group from 236 ± 14.2g to 341 ± 13.7g , at day 1 and 21 of gestation respectively. Two animals from the H group had 12 and 4 stillbirths ($P < 0.001$). There was a dose related (though non-significant) increase in the number of implantation sites, live pups (F_1), and female ratio per litter. External examinations of pups, soon after parturition, did not show any abnormalities.

Physical development of F_1 offspring:

Survival and body weight is shown in Table 2. All F_1 offsprings in the three groups survived until the end of the experiment, except of one male from the H group which died at 20 days of age. There was no significant difference between the groups in body weight at 2 days of age. Body weight gain, however, was less in the treated males and females up to 90 days of age. The body weight of treated animals was significantly less than that of the controls at 90 days of age, and was dose related in males but not so in females.

Other physical development parameters are shown in Table 3. There was a delay in ear and eye opening of L group at 2 and 14 days of age respectively. Hair growth of L and H offspring was advanced than that of the controls at 3 and 4 days of age respectively. There was no difference between the groups in time for tooth eruption, descent of testicles or vaginal opening.

Reflex and motor development:

Assessment of reflex and motor development of F_1 animals (Table 4) revealed a dose related loss of cliff avoidance at day 7 of age, the number of H (male + female) animals which backed away from the cliff, was significantly less than the controls. By 9 days of age, however, all animals in the three groups succeeded to perform the test. At day 13 of age, both males and females of L group, remained longer time hanging on horizontally stretched wire (forepaw grip stength). On the other hand, there was no difference between the groups in the number of animals that succeeded to turn over to right position within 15 seconds when placed on back on flat surface (surface righting) at 4-6 days of age, or when dropped, dorsal side downwards from height of 30 cm (mid-air righting) at 16-20 days of age, in straight line swimming and angle of body to surface during swimming at 5,8,11 and 14 days of age, and in ability to stay on a rotating rod at 30-33 days of age.

Sensory development:

The results are shown in Table 5. In the jumping test, pups were placed on a platform and allowed to jump from a low or high height to either the home cage or an empty cage at 10 and 21 days of age. At 10 days of age, when the eyes of pups were still close, about 60-70% of the pups in all groups jumped down from the low height to the home cage. The remaining pups, mostly did not leave the platform, with small number jumped down to the empty cage. At day 21 of age, when eyes were opened, 70-80% of pups jumped down to the home cage

from the low height. More of the H group pups jumped down from the high height than the controls at day 21 of age.

There was no significant difference between the groups in an auditory startle response at day 14 of age.

Behaviour of offspring:

Activity cage: Animals were tested in pairs (males or females) in an activity cage for one daily 5 min trial on 3 successive days at 39-50 days of age. The activity (in an arbitary scale) and number of fecal boluses deposited were recorded. The results show that males in both treated groups were hypoactive during the first trial (Table 6), while there was no difference between the groups in activity during the second and third trials (as a measure of habituation) or in the number of fecal boluses deposited (as a measure of emotionality) .

Head dipping test: At 70-75 days of age, the female offsprings were allowed a 2 min trial on the hole-board apparatus on 3 successive days. Male offsprings were not tested because of their large size at that age. The number of head dips into different holes (first dips, as a measure of exploration), or into same hole with no intervening locomotation (repeated dips, as a measure of repetitive behaviour), as well as fecal boluses deposited were recorded. There was no difference in exploration, repetitive behaviour or habituation between the groups. Females of H group, however, showed higher emotionality during the first trial, and those of L group showed less emotionality during the third trial (Table 6).

Conditioned avoidance learning: At 70-80 days of age each animal, after a familarisation period of 5 min, was given 20 conditioned stimuli (light/buzzer); unconditioned stimuli (electric shock to feet) pairings daily at 20 second intervals for 5 successive days. The number of successful avoidances and total waiting time (seconds) was recorded. Males showed a dose related decreases in

the number of successful avoidances (Table 7) with significantly increased waiting time.

Swimming maze: At 80-90 days of age, the animals were placed at start in central short arm of E-shape water-filled maze with escape ladder at end of right hand arm on first day, left hand arm on second day. The time taken to escape and errors made were recorded. The trials were run at 30 min intervals until 3 successive trials with no errors were performed. Males of L group took more time and made more errors to learn the maze on the second day (an opposite response to that previously learned). There was a tendency of the L and H females of making less errors to learn the maze on both days, the difference, however, was not significant (Table 7).

Biochemical functions:

When F_1 males were sacrificed at 120 days of age, after an overnight food deprivation, a dose related increase in serum cholesterol and decrease in serum GPT levels was observed. An increase in serum glucose (18%) and blood urea nitrogen (14%) was also observed in H groups. There was no significant difference between the groups in serum albumin, total proteins, total bilirubin, triglycerides, creatinine, alkaline phosphatase, or GOT levels (Table 8).

Fertility and reproductive function of F_1 rats:

Results are shown in Table 9. There was no significant difference between the groups in Fertility index of F_1 rats. The number of F_2 pups born alive to the treated groups was larger and significantly so for H group. There was a significant reduction in survival of L offspring compared to that of the controls. F_2 male offspring had matched body weight in the three groups, while females of treated groups were born heavier. Body weight gain of H male and L and H female offspring was significantly lower than that of the controls.

Physical development of F_2 offspring:

Results are shown in Table 10. Offspring of L rats had delayed ear opening at 3 and 4 days of age. Hair growth, tooth eruption and eye opening was advanced in both L and H groups than the control group. There was no difference between the groups in surface righting reflex.

DISCUSSION

The low (L) and high (H) doses used in the present experiment (given in the drinking water) were 4 and 8 times, respectively, that shown earlier to produce an anti-inflammatory activity when given orally to rats[10], and that of the maximal clinical dose of RA (4 g/ patient, daily), based on surface area ratio.

Administration of the extract was started 15 days before mating, through gestation and parturition, until day 25 post-partum. Therefore, treatment covered about 3 estrus cycles before mating to assess the effect on fertility of females. A low fertility rate (30-35%), however, was observed in all groups. In the present experiment, mating was allowed only for two hours each morning for 5 successive days. This procedure was followed, instead of caging females with males overnight or continuously for 5 days, to determine the time of mating with minimal variability, and to avoid non-specific changes in gestational period and body weight of pups.

Treatment had no effect on body weight gain of parental animals (F_0) during pregnancy or on length of gestation. Two animals from the H group had all their pups delivered dead, which could be due to difficulty in parturition. Examination of these stillbirths showed no obvious reason for their death.

A dose related (though non-significant) increase in the number of implantation sites, live pups (F_1), and female ratio per litter was observed. A similar effect was shown earlier in mice[12]. The effect on the number of implantations and litter size could be due to

maturation of more ovarian follicles with subsequent ovulation, and/or enhancement of implantation of the zygotes after fertilization. The RA extract was shown[12] to possess a slight but significant estrogen-like activity in both non-overiectomized and overiectomized mature mice. The latter effect could be induced by the steroided lactones (Withanolides), present in *Withania somnifera*[19], a major component of RA extract. The change in sex-proportion could be due to variation in vaginal and intracervical pH as a result of direct actions of the extract or through a hormone-mediated effect. Changes in pH levels affect the motility of spermatozoa containing X and Y chromosomes[20]. In man, male zygotes are formed earlier in the cycle than female zygotes[20, 21], and this is related to maternal hormone levels[22]. RA extract induced estrus cycle in mice[12], and this could mean that, at the time of mating, the treated animals were already late in the cycle which favours fertilization of female zygotes.

External examination of the pups soon after parturition did not show any malformation. This was supported by the finding that all offsprings born alive, survived until the end of the experiment, except one male pup from the H group. Neonatal mortality may be due to the offspring being anomalous in some subtle way[23], and these anomalous offsprings are usually destroyed by the mother[24]. Studies in mice, showed no association of RA treatment with malformations[12].

No effect on maternal body weight gain during gestation period, as well as on offspring birth weight was observed. That eliminates the possibility of undernutrition during gestation period as a result of giving the extract in the drinking water. Body weight gain of treated F_1 offspring during preweaning and upto 90 days of age, however, was less than that of the controls. This could be due to undernutrition during lactation period. It has been reported[25, 26] that, undernutrition during gestation results in low birth weight,

but by weaning "catch up" is observed and there is no difference in body weight of adults compared with controls. Undernutrition during lactation, however, results in low weaning body weight and no subsequent "catch up". In neither case is there any increase in pup mortality[25,26]. Therefore, the effect of treatment in the present experiment, is similar to undernutrition during lactation period; possibly due to decreased lactation or change in maternal behaviours towards their offsprings. The decrease in body weight gain might have influenced the observed delay in ear and eye opening of L pups at 2 and 14 days of age respectively. A similar effect, however, was not seen in the H group. No effect on other physical developmental parameters was observed.

Reflex and motor development:

These tests are designed to assess the rate of development of basic motor skills and fine motor co-ordination[14]. No differences between the groups were observed in reflex and motor development. Treatment had no effect on surface or mid-air righting, in swimming performance, or in ability to stay on a rotating rod. These tests were carried out from early days of life (day 4) upto 37 days of age. A loss of cliff avoidance of H animals was observed at day 7 of age. Cliff avoidance is a measure of maturity of forelimb development (pushing the body sideways or backwards when placed on the edge of a cliff or tabletop looking down), the stimulus being presumably tactile to the paws, nose and vibrassae[14]. Other tests for motor development did not show delayed forelimb maturation. Therefore, poor motivation of the test could result in the observed difference. The increase in grip strength of treated offspring is not dose related and can be influenced by the relatively lower body weight of these animals. It has been shown previously that, undernutrition of rat offspring during suckling period produced cerebellar growth retardation accompanied by motor

deficits[25,26]. In the present experiment, however, rat offspring had reduced body weight gain but without any signs of motor deficits or cerebellar retardation. That could be due to less severe under-nutrition in the present experiment campared to that reported earlier[25, 27].

Sensory development:

There was no difference between the groups in an auditory startle response which measures development of functional hearing. Olfaction and visual ability were measured in a jumping-down test. The test was performed at day 10 of age, when the eyes of the pups were still closed, to measure olfaction (heat sensation could also be involved). The results showed no difference between the groups at that age. At day 21 of age, when the eyes were opened, the test can measure visual ability[14]. In the present experiment, more of H group pups jumped down from the high height than the controls at 21 days of age. This may indicate a visual deficit (depth perception deficit), abnormally high motivation to join litter mates, or lack of fear of jumping from heights.

Behaviour:

Activity, emotionality and habituation[28] were assessed using the activity cage. Animals were used in pairs and tested for 5 min daily trial on 3 successive days. Treated males were hypoactive during the first trial. Exploration, repetitive behaviour, emotionality and habituation, were also assessed on a hole board apparatus[15]. Females were tested singly for 2 min daily trial on 3 successive days. It was asssessed on a hole board because they were large in size at the time of testing (70 days of age). H animals showed higher emotionality during the first trial and L animals showed lower emotionality during the third trial. Differences in emotionality became clear when the animals were tested individually using the hole board, than when tested in pairs using the

activity cage. Unfamiliar situation induces fear, due to anxiety exhibited in animals when placed in a new environment.

Learning ability of the offspring were tested using conditioned avoidance and swimming maze. In both tests, treated males were slower, while treated females were faster to learn both tasks. These results may be difficult to interpret, but the observed sex difference in learning ability may emphasise the influence of extract administration during vulnerable periods of development on the hormonal status of rat offspring. The extract was found to have a weak estrogenic and androgenic activity in female and male mice respectively[12].

Biochemical functions:

When male offsprings (F_1) were sacrificed at 120 days of age, a slight but significant increase in blood glucose and urea nitrogen, as well as, a decrease in GPT levels were observed in the H group. These changes may indicate an elevation of blood glucose through promotion of gluconeogenesis from amino acids, and decreased carbohydrate utilization[29]. The increase of blood urea nitrogen without a concomitant increase of creatinine may indicate a case of mild prerenal azotemia in these rats[30].

Productive performance of offspring (F_1):

There was no significant difference between the groups in fertility. The number of live pups (F_2) born to the treated offspring (F_1) was significantly higher, an effect which is similar to that observed in the parental (F_0) animals. The survival of F_2 offspring in the L group was slightly lower at day 21 of age. Again, similar to F_1 rats, L and H, F_2 offspring were born with virtually similar body weight. Their body weight gain, however, was lower than that of the controls up to day 21 of age, which may indicate some undernutrition during lactation period.

The results of the present study may indicate that administration of RA herbal extract before and during pregnancy and lactation periods, did not produce any structural malformations in rat offspring. It had no effect on fertility of parental rats or of their offspring, reflex and motor development and sensory development of F_1 offspring. The significant findings observed, were an increase in the number of live pups born to the parental and F_1 rats, an increase in the female ratio of F_1 rats, a decrease in body weight gain of both F_1 and F_2 rats, as well as, changes in emotionality, learning ability and biochemical functions. These results may support an earlier view[31], that postnatal functions does, at least in some cases, appear to be more sensitive indicator of teratogenic effects than physical malformations.

According to the suggestion of Rodier[17] that, an agent which affects a functional alteration must be regarded as hazardous, even if it cannot be shown to produce morphological changes, and based on the findings of the present study, it is advisable, therefore, not to give these herbal agents to women during pregnancy or lactation period.

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TABLE 1: EFFECT OF THE HERBAL EXTRACT (RA) GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION TO PARENTAL (F0) FEMALE RATS, ON FERTILITY, GESTATION AND LITTER SIZE.

	Control	RA(L)	RA (H)
		400 mg/rat	800 mg/rat
No. of animals treated	20	20	20
No. of positive smears	13	14	10
No. of animals pregnant at term	7	7	6
Gestation period (days ± s.e.m)	23.4 ± 0.29	23.4 ± 0.3	23.3 ± 0.33
Total no. of implantations / no. of dams[a]	60/9	60/8	60/6
No. of live pups/ no. of dams (%)	41/7 (68)	47/7 (78)	35/4 (58)
Mean no. of live pups ± s.e.m.	5.86 ± .0.96	6.71 ± 0.7	8.75 ± 1.1
No. of males (%)	23 (56)	25 (53)	13 (37)
No. of females(%)	18 (44)	22 (47)	22 (63)

a: Implementation sites were counted, when all animals were killed at the end of the treatment period (25 days post-partum), 2 and 1 animals of control and L (low-dose)groups respectively had implantation sites and no live pups, 2 animals of H (high-dose) group had 12 and 4 stillbirths ($P < 0.001$) and no live pups.

TABLE 2: EFFECT OF THE HERBAL EXTRACT (RA) GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST-PARTUM TO PARENTAL (F0) FEMALE RATS ON SURVIVAL AND BODY WEIGHT OF THEIR OFFSPRING (F_1) UPTO 90 DAYS OF AGE

Day of Age	Males			Females		
	Control	RA (L)	RA (H)	Control	RA (L)	RA (H)
	(22)	(23)	(10)	(16)	(22)	(20)
2	8.28 ± 0.13	8.37 ± 0.19	8.15 ± 0.41	7.75 ± 0.10	7.4 ± 0.13	8.04 ± 0.23
7	18.65 ± 0.47	18.42 ± 0.65	16.69 ± 0.77*	17.36 ± 0.47	15.65 ± 0.18**	17.29 ± 0.49
15	37.9 ± 0.98	35.4 ± 0.81	31.4 ± 1.28**	33.2 ± 1.03	31.0. ± 0.68	32.3 ± 1.43
21	49.8 ± 1.1	50.1 ± 1.2	42.9 ± 2.2**	48.8 ± 1.14	44.5 ± 1.5	45.0 ± 2.21
30	95.1 ± 1.9	93.0 ± 1.5	81.6 ± 2.4**	85.8 ± 2.1	84.2 ± 1.71	81.2 ± 2.3
60	260.6 ± 4.4	264.2 ± 3.5	252.8 ± 8.0	193.2 ± 4.4	175.1 ± 3.8**	180.5 ± 1.8**
90	286.0 ± 4.64	264.4 ± 4.95**	259.7 ± 8.7**	202.9 ± 4.78**	176.6 ± 5.85**	178.7 ± 3.86**

Number of offspring at day 2 of age, after culling, is shown in parentheses, and one male from H group died at day 29 of age. Results are expressed as mean body weight g + s.e.m. and compared using Student's 't' test.

*$P<0.05$ **$P<0.01$

TABLE 3 : EFFECT OF THE HERBAL EXTRACT (RA) GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST-PARTUM TO PARENTAL (F0) FEMALE RATS ON PHYSICAL DEVELOPMENT OF THIER OFFSPRING (F_1)

Number of offspring	Day of age	Control 38	RA (L) 45	RA (H) 30
Ear opening	2	35 (92.1)	30** (66.7)	28 (93.3)
-	3	38 (100)	45 (100)	30 (100)
Hair growth	3	0 (0.0)	7** (15.6)	0 (0.0)
	4	2 (5.3)	7 (15.6)	8** (26.7)
	5	38 (100)	45 (100)	30 (100)
Tooth eruption	9	2 (5.3)	1 (2.2)	3 (10.0)
Lower incisors	10	18 (47.4)	19 (42.2)	11 (36.7)
	11	35 (92.1)	33 (73.3)	24 (80.0)
	12	38 (100)	45 (100)	29 (96.7)
Eye opening	14	17 (44.7)	10* (22.2)	18 (60.0)
	15	38 (100)	26 (57.7)	23 (76.7)
	16	38 (100)	44 (97.8)	30 (100)
Descent of testicles	27	22 (100)	23 (100)	10 (100)
Vaginal opening	30	0 (0.0)	3 (13.6)	0 (0.0)
	36	8 (50.0)	15 (68.2)	13 (65.0)
	42	15 (93.8)	22 (100)	20 (100)

Results are expressed as the number of offspring with positive findigns (%) and compared using Chi-square test – *: $P < 0.03$ **:$P < 0.01$

TABLE 4: EFFECT OF THE HERBAL EXTRACT (RA) GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST-PARTUM TO PARENTAL (F0) FEMALE RATS ON REFLEX AND MOTOR DEVELOPMENT OF THEIR OFFSPRING (F_1).

Number of offspring	Age (days)	Control 38	RA (L) 400 mg/rat 45	RA (H) 800 mg/rat 30
-Surface righting:	4	34 (89.5)	38 (84.4)	28 (93.3)
	5	38 (100)	42 (93.3)	30 (100)
-Cliff avoidance:	7	36 (94.7)	38 (84.4)	22 (73.3)*
	8	35 (92.1)	39 (86.7)	24 (80.0)
-Swimming performance:				
(A) Swimming at 30°angle of body	5	27 (71)	35 (78)	21 (70)
to surface	14	38 (100)	43 (96)	29 (97)
(B) Straight line swimming	5	8 (21)	17 (38)	2 (6.7)
	14	24 (63)	45 (100)	21 (70)
-Grip strength,[a]	♂ 13	12.2 ± 1.67 (22)	19.8 ± 2.35 (23)*	14.6 ± 2.22 (10)*
	♀ 13	10.3 ± 1.21 (16)	16.6 ± 2.05 (22)*	14.6 ± 1.89 (20)*
-Mid-air righting	16	23 (60.5)	27 (60)	16 (53.3)
	18	35 (92.1)	37 (82.2)	26 (86.7)
	20	37 (97.4)	44 (97.8)	28 (93.3)
Rotarod:				
1st trial (8 r.p.m)	30-33	12 (31.6)	19 (42.2)	12 (41.4)
2nd trial (12 r.p.m)		21 (53.3)	29 (64.4)	14 (48.3)
3rd trial (16 r.p.m)		27 (71.1)	37 (82.2)	18 (62.1)

Results are expressed as number of animals succeeded to perform the test (%), and compared using Chi-square test.

a. Results are expressed as mean time (sec) taken until fall off the rod ± s.em. (Number of animals in respective groups are shown at the top of each column).

* $P < 0.02$

TABLE 5: EFFECT OF THE HERBAL EXTRACT (RA) GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST-PARTUM TO PARENTAL (F0) FEMALE RATS ON SENSORY DEVELOPMENT OF THEIR OFFSPRING (F_1)

Number of offspring	Day of age	Control 38	RA (L) 45	RA (H) 30
Jumping test:				
a. No. of pups jumping down to the home cage	10	24 (63.2)	33 (73.3)	22 (73.3)
from low height (10 cm).	21	26 (68.4)	38 (84.4)	25 (83.3)
b. No. of pups jumping down to the empty cage	10	2 (5.2)	4 (8.4)	2 (6.7)
from low height (10 cm).	21	3 (7.9)	2 (4.5)	3 (10.0)
c. No. of pups jumping down to either cage from	10	6 (13.2)	14 (26.7)	4 (13.3)
high height (40 cm).	21	11 (10.5)	17 (28.9)	17* (40.0)
Auditory startle	14	38 (100)	40 (88.9)	30 (100)
	15	38 (100)	43 (95.6)	30 (100)

Results are expressed as the number of offspring with positive response (%) and compared using Chi-square test *: $P < 0.02$

TABLE 6: EFFECT OF THE HERBAL EXTRACT (RA), GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST- PARTUM TO PARENTAL (F0) FEMALE RATS, ON BEHAVIOUR OF THEIR OFFSPRING (F_1) USING ACTIVITY CAGE AND HOLE BOARD AT 35 AND 70 DAYS OF AGE RESPECTIVELY.

	First trial	Second trial	Third trial
Activity cage:			
Control males (n = 10 pairs)	377 ± 39.5	335 ± 48.4	330 ± 27.3
RA (L) males (n = 9 pairs)	266 ± 28.3*	210 ± 36.6	210 ± 21.9
RA (H) males (n = 3 pairs)	261 ± 17.9	229 ± 99.0	289 ± 75.9
Control females (n = 6 pairs)	284 ± 34.3	278 ± 69.2	245 ± 56.8
RA (L) females (n = 10 pairs)	268 ± 18.7	195 ± 20.7	242 ± 35.9
RA (H) females (n = 9 pairs)	325 ± 28.6	343 ± 53.4	268 ± 34.3
Hole board:			
a. Mean no. of first dips			
Control females (n = 16)	9.9 ± 0.88	8.5 ± 1.11	7.3 ± 0.93
RA (L) females (n = 22)	11.2 ± 0.99	8.6 ± 0.93	6.5 ± 0.91
RA (H) females (n = 20)	9.8 ± 0.83	10.3 ± 1.45	7.1 ± 0.93
b. Total no. of repeated dips/ no. of animals:			
Control females (n = 16)	8/4	8/8	1/1
RA (L) females (n = 22)	6/5	10/7	3/3
RA (H) females (n = 20)	12/5	12/7	4/4
c. Total no. of fecal deposits/ no. of animals:			
Control females (n = 16)	3/1	3/1	19/6
RA (L) females (n = 22)	12/6	1/1	6/1**
RA (H) females (n = 20)	17/7*	6/2	20/6

Activities of animals in the activity cage are measured on an arbitrary scale and expressed as mean ± s.e.m for each pair of animals and compared using Student's 't' test.
Results in b and c are compared using Chi-square test: * $P < 0.05$; ** $P < 0.01$

TABLE 7: EFFECT OF THE HERBAL EXTRACT (RA), GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST- PARTUM TO PARENTAL (F_0) FEMALE RATS ON LEARNING ABILITY OF THEIR OFFSPRING (F_1) AT 70-90 DAYS OF AGE.

	Control	RA (L) 400 mg/rat	RA (H) 800 mg/rat
Conditioned learning:			
Total no. of avoidances (males) n =	7	7	6
1st day (%)	5 (3.6)	2 (1.4)	0 (0.0)
3rd day (%)	14 (10.0)	11 (7.9)	2 (1.7)
5th day (%)	33 (23.6)	24 (17.1)	8 (6.7)*
Total no. of avoidances (females) n =	6	7	4
1st day (%)	2 (1.7)	4 (2.9)	4 (5.0)
3rd day (%)	7 (5.8)	10 (7.1)	9 (11.3)
5th day (%)	4 (3.3)	47 (33.6)*	18 (22.5)*
Swimming maze:			
a. Males n =	14	14	9
Mean time (sec) taken to learn (right)	144.6 ± 9.99	242.3 ± 28.1**	109 ± 14.8
Mean no. of errors to learn (right)	5.57 ± 0.05	7.43 ± 0.58*	6.1 ± 0.95
Mean time (sec) taken to learn (left)	137.7 ± 23.8	121.7 ± 16.1	154 ± 23.1
Mean no. of errors to learn (left)	5.93 ± 0.74	6.0 ± 0.71	8.44 ± 0.44*
b. Females n =	7	8	8
Mean time (sec) taken to learn (right)	140.0 ± 15.7	110 ± 22.6	174 ± 32.1
Mean no. of errors to learn (right)	7.57 ± 1.02	5.88 ± 1.06	6.38 ± 1.07
Mean time (sec) taken to learn (left)	246 ± 117.6	134.4 ± 32.6	64.3 ± 23.3
Mean no. of errors to learn (left)	6.71 ± 0.89	5.0 ± 1.07	4.5 ± 1.05

Results are compared using Chi-square test (conditioned learning), and Student's 't' test (swimming maze). n = number of animals * $P < 0.02$; ** $P < 0.001$

TABLE 8: EFFECT OF THE HERBAL EXTRACT (RA), GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST- PARTUM TO PARENTAL (F_0) FEMALE RATS ON BIOCHEMICAL FUNCTIONS OF THEIR MALE OFFSPRING (F_1) AT 120 DAYS OF AGE.

Number of animals	Control 14	RA (L) 400 mg/rat 13	RA (H) 800 mg/rat 9
Glucose mmol/L	4.43 ± 0.22	4.55 ± 0.18	5.22 ± 0.27*
Blood urea mitrogen (BUN) mmol/L	4.9 ± 0.17	5.20 ± 0.27	5.60 ± 0.23*
Albumin G/L	9.9 ± 0.29	8.8 ± 0.26	9.1 ± 0.30
Total proteins G/L	62.6 ± 0.75	60.7 ± 0.6	60.0 ± 1.70
Total bilirubin mmol/L	2.1 ± 0.13	2.3 ± 0.75	2.0 ± 0.20
Cholesterol mmol/L	1.11 ± 0.05	1.27 ± 0.05*	1.3 ± 0.04**
Triglycerides mmol/L	0.54 ± 0.04	0.47 ± 0.03	0.58 ± 0.23
Creatinine mmol/L	31.0 ± 1.3	29.0 ± 2.2	31.0 ± 3.0
Alkaline phosphatase U/L	100.0 ± 7.75	110.0 ± 5.4	99.0 ± 3.6
GOT U/L	104 ± 3.1	105.0 ± 2.4	106.0 ± 4.7
GPT U/L	50.3 ± 2.1	40.7 ± 1.3**	39.1 ± 3.8**

Results are expressed as mean ±s.e.m and compared using Student's 't' test
*P < 0.03; ** P < 0.01

TABLE 9: EFFECT OF THE HERBAL EXTRACT (RA), GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST- PARTUM TO PARENTAL (F0) FEMALE RATS ON FERTILITY AND LITTER SIZE OF THEIR OFFSPRING (F_1), AS WELL AS ON SURVIVAL AND BODY WEIGHT OF THE SECOND GENERATION (F_2) DURING THE PREWEANING PERIOD.

	Control	RA (L) 400 mg/rat	RA (H) 800 mg/rat
No. of pregnant/No. mated (1st trial)	5/16 (31.3)	12/22 (54.5)	11/18 (61.1)
No. of pregnant/No . mated (2nd trial)	8/11 (72.7)	1/10 (10.0)	8/9 (88.9)
Total No. of pregnant/No. mated (%)[a]	13/16 (81.3)	13/22 (59.1)	19/20 (95.0)
Mean no. of live fetuses + s.e.m.	5.62 ± 0.77	7.23 ± 0.82	7.53 ± 0.46*
No. of stillbirths (%)	3 (3.95)	0	4 (2.30)
No. of males at day 1 of age (%)	42 (57.5)	51 (54.3)	73 (51.0)
No. of females at day 1 of age (%)	31 (42.5)	43 (45.7)	70 (49.0)
Total no. of offspring at day 21 of age (survival, %)	65 (89)	68 (72.3)	134 (93)
Body weight of males at day 2 of age	7.52 ± 0.24	7.46 ± 0.15	7.52 ± 0.18
Body weight of males at day 21 of age	38.1 ± 1.18	39.3 ± 2.12	32.6 ± 0.98 **
Body weight of females at day of 2 of age	6.95 ± 0.30	7.47 ± 0.18	7.60 ± 0.16*
Body weight of females at day of 21 of age	36.4 ± 1.48	31.2 ± 1.31*	33.8 ± 1.13

a. Fertility index * : $P < 0.05$ ** : $P < 0.01$

TABLE 10: EFFECT OF THE HERBAL EXTRACT (RA), GIVEN 15 DAYS BEFORE MATING AND THROUGH GESTATION AND PARTURITION UNTIL DAY 25 POST- PARTUM TO PARENTAL (F0) FEMALE RATS ON PHYSICIAL DEVELOPMENT OF THE SECOND GENERATION (F_2) DURING PREWEANING PERIOD.

	Day of age	Control	RA (L) 400 mg/rat	RA (H) 800 mg/rat
Ear opening	3	54/65 (83.1)	47/94 (50.0)**	118/141 (83.7)
	4	65/65 (100)	81/94 (86.2)**	140/141 (99.3)
Hair growth	3	16/65 (24.6)	17/94 (18.1)	72/141 (51.1)**
	4	40/65 (61.5)	69/94 (73.4)	122/141 (86.5)**
	5	58/65 (89.2)	85/86 (98.8)**	136/140 (97.1)*
Tooth eruption	9	6/65 (9.2)	11/69 (15.9)	9/134 (6.7)
	10	21/65 (32.3)	35/69 (50.7)*	51/134 (38.1)
	11	30/65 (46.2)	50/69 (72.5)**	88/134 (65.7)**
	12	52/65 (80.0)	61/69 (88.4)	108/134 (80.6)
Eye opening	14	9/65 (13.8)	11/69 (15.9)	32/134 (23.9)
	15	21/65 (32.3)	46/69 (66.7)**	100/134 (74.6)**
	16	39/65 (60.0)	50/69 (72.5)	117/134 (87.3)**
	17	60/65 (92.3)	63/69 (91.3)	132/134 (98.5)
Surface righting	4	61/65 (93.8)	84/94 (89.4)	126/141 (89.4)
	5	62/65 (95.4)	85/86 (98.8)	139/140 (99.3)

Results are expressed as the number of offspring with positive findings/total number (%) and compared using Chi-square test. * $P < 0.05$; ** $P < 0.01$

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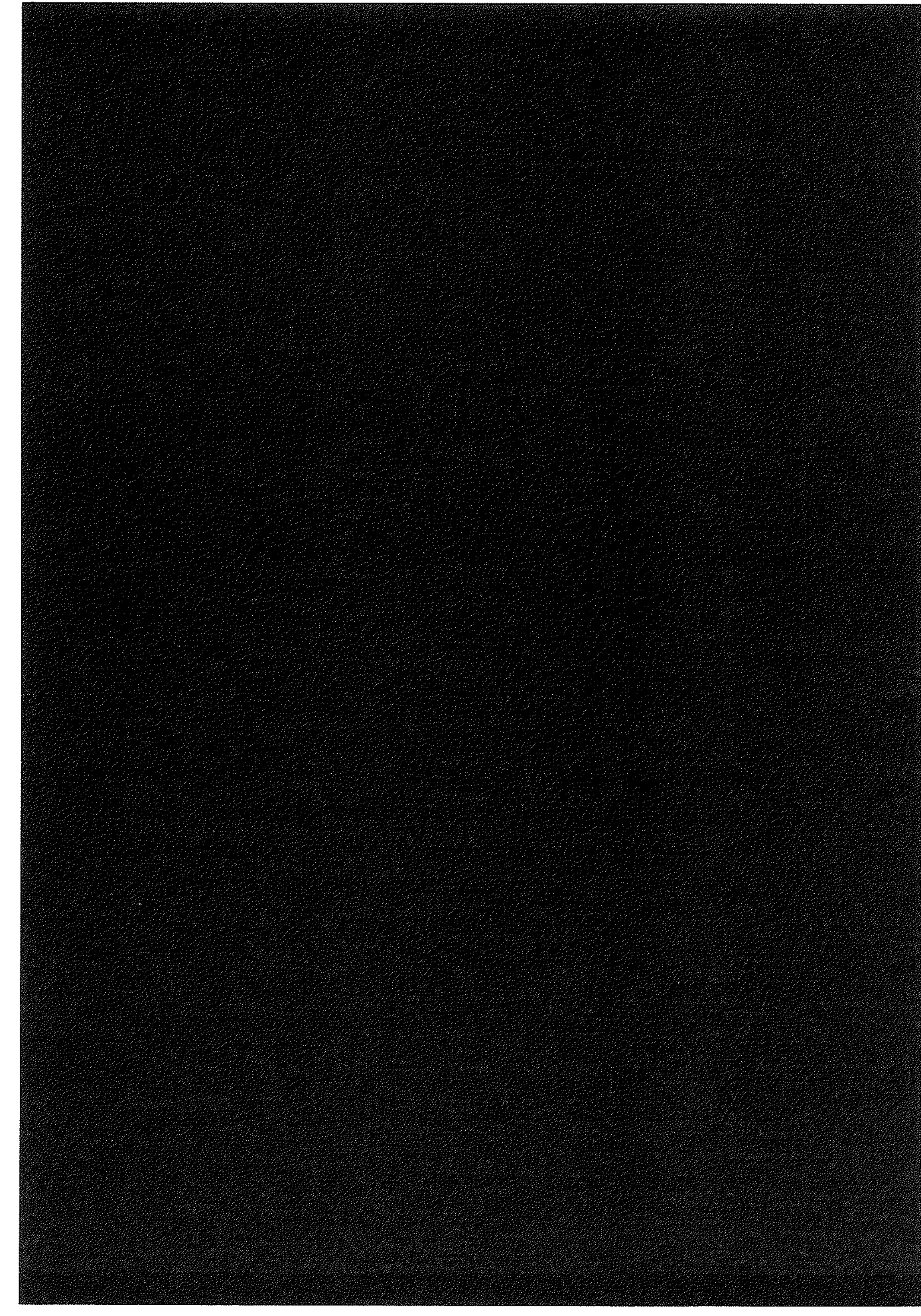
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THE BIO-ACTIVITY OF CERTAIN MEDICINAL PLANTS ON THE STABILIZATION OF RBC MEMBRANE SYSTEM

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INTRODUCTION

Inflamation is a complex phenomenon. It is one of the fundamental responses of cells and tissues to injury caused by noxious and infectious agents. A vast array of substances known as mediators are formed or released either concurrently or successively at the site of injury from various plasma or cell sources in response to an etiological factor[1]. Anti-inflammatory agents exert their effect through a spectrum of different mode of actions[2]. All the steroidal and non-steroidal anti-inflammatory drugs currently available are probably polycompetent in that they are able to modulate more than one mediator or cellular events concerned with inflammatory response[3]. Lysosomes are packed with hydrolytic enzymes. When leucocytes phagocytize an inflammatory agent, they release lysosomal hydrolases which damage the surrounding tissues[4]. Glucocorticoids and several aspirin like drugs have been shown to stabilize lysosomes and this may account for one of their major mechanisms of action[5,6]. It has been reported that since RBC membrane has resemblance to lysosomal membrane, the effect of drugs on stabilization of RBC membrane could be extrapolated to stabilization of lysosomal membrane[7]. The effect of anti-inflammatory drugs including herbal drugs on the stabilization of RBC

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membrane system subjected to either hypotonic or thermic or their combined stresses have been extensively studied[8-15],

Some of herbal formulae are used successfully in the treatment of human rheumatoid arthritis (RA formula) and allergic rhinitis (AR formula) at Islamic Centre for Medical Sciences, Ministry of Public Health, State of Kuwait. Formula RA and its components- *Withania somnifera* roots, *Pyrethrum indicum* roots, *Merendra persica* corm and *Alpinia galanga* rhizome (RA composition: 2: 1: 1: 1 in the above order) were reported to exert anti-inflammatory activity in both acute and chronic inflammation[16]. Similarly AR formula (used for treating allergic rhinitis) having a composition of *Cydonia oblangata* seed, *Ziziphus valgaris* fruits and *Cordia latifolia* fruits (3: 5: 6 respectively) was found to be anti-inflammatory and anti-histaminic (unpublished). With a view to understand the mechanism of action of these formulae (RA and AR) and its components, membrane stabilization studies using sheep RBC subjected to a combined hypotonic and heat stresses were undertaken.

EXPERIMENTAL

Preparation of RBC system:

Sheep blood was collected from slaughter house at Kuwait in sterile Alsever's solution (containing 2% dextorse, 0.8% sodium citrate,, 0.05% citric acid and 0.42% sodium chloride stored at 4°C). Fresh sheep blood and the blood stored in refrigerator at 4°C for 24-72 hours were used for preparing sheep RBC. Blood was centrifugated at 2000 rpm for 10 min and then supernatant was discarded. The cells were suspended in isosaline (0.85% NaCl solution) and recentrifuged. After removing the supernatant, 10% cell suspension was prepared using isosaline. In case of unfresh RBC, the cells that responded uniformly in triplicates to heat and osmotic stresses were used.

Preparation of isosaline extracts of the herbal formulae and their components:

Five gm of each of the test materials (RA formula; *Alpinia galanga* - rhizome; *Withania somnifera*- roots; *Pyrethrum indicum* - roots; *Merendra persica* - corm; AR formula; *Cydonia oblongata* - seeds; *Ziziphus vulgaris* - fruits and *Cordia latifolia* - fruits) were suspended in 100 ml of 0.85% NaCl solution in conical flasks and kept in the shaking metabolic shaker with a speed of 75 strokes/min for 6 hr. The flasks were kept at room temperature overnight in dark. The extracts were centrifuged in cold and filtered. The clear filtrate was used after adjusting the pH to 7 - 7.4. Similarly, standard drugs such as acetyl salicylate (1 mg/ml) and diphenhydramine hydrochloride (1 mg/ml) were prepared in isosaline after adjusting the pH to 7 - 7.4.

Effect of various drugs on hypotonic saline and heat induced sheep RBC lysis[8,9,15]*:*

The incubation mixture consisted of 2 ml hyposaline (0.25% or 0.36% NaCl solution), 1 ml 0.15 M phosphate buffer (pH 7.4), different concentrations of drugs made up to 1 ml with isosaline and 0.5 ml of 10% sheep RBC suspension. Similarly, drug control was put up with isosaline instead of SRBC. They were incubated at 56°C for 30 min, cooled in running tap water and centrifuged at 1500 rpm. The supernatant was measured at 560 nm for haemoglobin release. Control had 1 ml 0.85% saline instead of drug. The control represents 100% lysis. The membrane stabilization activity was calculated as follows:

Percentage membrane stabilization activity

$$= \frac{100 - (\text{Drug test value} - \text{Drug control value}) \times 100}{\text{control value}}$$

The value represents the average of triplicates ± s.e.m.

In case of studies on LDH leakage from SRBC, the assay system was incubated with different concentrations of RA isosaline

extract and acetyl salicylate in presence of hyposoline (0.36% NaCl solution) and 0.1 M phosphate buffer (pH 7.4) at 37°C for 30 min. LDH activity in the supernatant after centrifuging the incubated assay system, was measured using ACA SX Dupont autoanalyser (USA) and haemoglobin was also measured at 560 nm in the supernatant. Control had 1 ml isosaline instead of drug with same composition as described earlier.

RESULTS AND DISCUSSION

Lysosomes are granules containing acid hydrolases which can be released by membrane disruptive agents. The discharge of these hydrolytic enzymes from lysosomes may be responsible for a variety of disorders that affect extracellular connective tissue components. Thus, abnormal fragility of lysosomes and increased extracellular activity of lysosomal enzymes have been implicated in a number of pathological phenomena involving inflammatory processes including human rheumatoid arthritis[17-19].

The ability of anti-inflammatory drugs to stabilize the lysosomal membrane and to inhibit lysosomal enzyme release have been investigated[20-22]. Anti-inflammatory agents such as indomethacin, phenylbutazone and flufenamic acid have been shown to inhibit hydrolase release from liver lysosomes[23, 24].

The compounds stabilizing lysosome membrane inhibit the release of enzyme content and occurrence of inflammation[25]. Reinhart[26] has reported the beneficial effect of bioflavonoids in rheumatoid arthritis. Flavonoids (rutin and tri-hydroxy ethyl rutin) exert stabilizing effect on the lysosomes *in vitro*[27] and (+)-Cyanidanol -3(+)-catechin *in vivo* in rats[28]. Hence anti-inflammatory activity of drugs may be exerted through stabilization of lysosomal membrane.

Many workers have used RBC system to establish the membrane stabilizing activity of drugs[8-15] since RBC membrane resembles lysosomal membrane[7].

Glenn and Bowmann[29] have found that lysis produced by a combination of heat and hypotonicity seems to be more sensitive than produced by either condition alone. Hence, in the present study, a combination of heat and osmotic stresses was chosen to produce the lysis of sheep erythrocytes.

When fresh sheep RBC was used in the present investigation, a biphasic response was observed i.e. a particular concentration offered maximal protection against osmotic and heat induced lysis of SRBC and higher concentrations of drugs caused lysis. From Fig. 1, it can be noted that AR extract (used for treating allergic rhinitis) showed a maximal membrane stabilizing activity (59.3%) at a concentration of 12.5 mg in the test system whereas RA extract (used for treating rheumatoid arthritis) gave a maximal response (47.74%) at a concentration of 24.9 mg in the test system. Diphenhydramine hydrochloride, an anti-allergic compound[30] exerted a maximal protection (50%) at a concentration of 500 µg in the test system whereas acetyl salicylate in a similar concentration exhibited weaker membrane stabilization activity (8.27%). In these studies, 0.25% hyposaline and 0.15 M phosphate buffer were used[31].

A number of lipid soluble non-steroidal anti-inflammatory drugs prevent hypotonicity induced haemolysis but they share the property with *beta* adrenolytics[32], local anesthetics[33], tranquilizers[33] and anti-histaminics[34]. Colchicine was also found to stabilize hypotonicity induced lysis of amphibian erythrocyte membrane[35]. The earlier report[36] suggested that the low concentrations (10^{-8}-10^{3}M) of the lipid soluble or surface active compounds protect the membrane from osmotic, mechanical or acid lysis but higher concentrations (about 10^{-4} - 10^{-3}M) produce lysis of membrane. It

was also reported by several workers that alcoholic fraction of some anti-inflammatory medicinal plants and indomethacin showed a biphasic activity in hypotonicity induced HRBC lysis[10,14,30].

Similarly, AR formula is found to have anti-inflammatory and anti-histaminic activity (unpublished). In the present investigation, membrane stabilization activity of RA and AR formulae may suggest the possibility that the anti-inflammatory activity of these formulae may be through the stabilization of lysosomal system. Further, diphenhydramine hydrochloride, an anti-allergic compound, showed a biphasic membrane stabilization activity in the present investigation like anti-histamines[34].

From Fig. 2, it can be noted that AR formula and two of its components *Ziziphus vulgaris* and *Cordia latifolia* showed a biphasic response against heat and hyposaline induced RBC lysis in case of unfresh RBC where same composition of phosphate buffer 0.15 M and hyposaline 0.25% was maintained. It is interesting to observe that similar concentrations of AR extract (about 12.5 mg) showed the highest activity in both cases of fresh and unfresh RBC system. However, the higher percentage of membrane stabilization activity for this concentration was noticed when unfresh erythrocytes were used. *Cydonia oblongata* did not show any membrane stabilizing activity.

It was reported that low concentrations of reserpine, tranquilizers and local anaesthetics reduce the release rate of catecholamines from chromaffin granules but accelerate release rate at higher concentrations[37,38]. The mechanism of stabilization of erythrocyte membrane was attributed to the fact that these compounds increase critical haemolytic volume (Vc) of erythrocyte[39]. This might be due to an actual increase in membrane area or change in membrane viscoelasticity. At sublytic concentrations, these drugs overexpand the membrane and increase the permeability which results in lysis of cells. AR formula has been found to possess anti-flammatory and

anti-histaminic activities (unpublished). The membrane stabilization activity of AR formula may play a role in its anti-inflammatory activity.

The membrane stabilization activity of AR formula may also be involved in its anti-histaminic activity. It is well known that many anti-allergic agents can prevent histamine release from rat mast cells[40]. Ennis et al[41] have postulated that prevention of histamine release from sensitized rat mast cells under exposure to specific antigen may be due to the stabilization of membrane of mast cells. Similarly, Akagi et al[40] suggested that NCO - 650, a new anti-allergic agent which prevents histamine release induced by compound 48/80 may promote this activity by stabilization of membrane system. So, it is possible that anti-histaminic activity of AR formula may be due to membrane stabilization effect.

From Fig. 3, it can be noted that isosaline extract of RA formula at a concentration of 49.8 mg showed maximal SRBC stabilization activity when unfresh SRBC system was used and the effect was dose dependent. This activity at this concentration was the highest (96.8%) among its components and acetyl salicylate (1 mg). *Pyrethrum indicum* at this concentration showed 93.5% activity and its lowest concentration (12.45 mg) exerted higher activity (79%) than RA formula and its other components at a similar concentration. After reaching 90% activity at a concentration of 24.9 mg, the membrane stabilization activity of *Pyrethrum indicum* did not increase proportionately with further increasing concentrations. The isosaline extract of *Withania somnifera* exhibited a maximal membrane stabilizing activity of 88.9% at a concentration of 49.8 mg. This drug also showed a dose dependent membrane stabilization activity and the activity showed a steep rise in the first two concentrations. There was not much difference between second and third concentrations. *Merendra persica* evoked the highest activity of 86.6% at a concentration of 49.5 mg. There

were sharp rises in the first two concentrations followed by slow rises in the last two concentrations. *Alpinia galanga* was similar to *Merendra persica* in the concentration membrane stabilization activity profile but the magnitude was smaller than that of the latter. It showed the highest activity of 73.8% at a concentration of 48.6 mg. Membrane stabilization activity profile for acetyl salicylate was resembling that of *Withania somnifera* with much lesser activity in all the concentrations, the highest activity being 46.9% at a concentration of 1 mg.

It is interesting to note that unfresh SRBC system exhibited the higher membrane stabilization response for RA formula, AR formula and acetyl salicylate compared to fresh SRBC. It is suggested that non-steroidal anti-inflammatory drugs interact with biological membranes, the proteins being main binding sites for these drugs in biological membrane[42] *in vitro*. Hence, in case of unfresh SRBC, there is the possibility of changes in the fluidity of membrane system and so, more accessibility and better binding of RA formula, AR formula and acetyl salicylate with proteins of membranes of unfresh SRBC so that higher activity has been observed. Report on binding of several membrane active drugs to albumin and also on binding of these drugs to erythrocyte membrane using a fluorescent probe, 8-anilino - 1- naphthalene sulphonate is available[43]. Anti-inflammatory activity of several agents has been tested *in vitro* on the basis of prevention of heat induced albumin denaturation by anti-inflammatory drugs[44-46]. We too have found that aqueous extract of RA formula, its components viz. *Withania somnifera, Alpinia galanga, Pyrethrum indicum, Merendra persica,* AR formula and acetyl salicylate are able to prevent heat induced bovine serum albumin denaturation (unpublished). This observation is in concurrence with the present results on SRBC stabilization against hypotonicity and heat induced membrane lysis by these drugs. A significant role of erythrocyte

proteins in the maintenance of structural integrity of erythrocyte membranes has been cited[47]. Hence SRBC membrane stabilizing effects of isosaline preparations of currently tested drugs may be due to the binding of active principles of herbal drugs with proteins of SRBC membranes.

Lactate dehydrogenase (LDH) in erythrocyte is involved in the glycolytic pathway. Its molecular weight is 1,30,000[48] whereas molecular weight of haemoglobin is 68,000[49]. it has been reported that during the stage of haemolysis, there are breaks or holes in membranes as shown by electron microscopy[50]. Conventionally, haemoglobin has been selected as the marker for studying the leaky nature of RBC membrane system[8]. We were interested in knowing whether LDH, an intra-cellualr metabolic enzyme having higher molecular weight than haemoglobin is released by hypotonicity induced RBC lysis and if so, whether RA formula and acetyl salicylate are able to prevent LDH leakage. For this purpose, LDH activity and haemoglobin concentrations were measured simultaneously. From Fig. 4, it can be noted that when unfresh RBC subjected to different hypotonic stresses including distilled water was incubated at 37°C for 30 min, distilled water treatment released maximally LDH and haemoglobin from SRBC followed by 0.36% NaCl solution and other concentrations of NaCl solutions. There was a close similarity between LDH leakage and haemoglobin release from SRBC depending upon hypotonicity. Similarly, when unfresh RBC was incubated with different concentrations of isosaline extracts of RA formula and acetyl salicylate in hyposaline medium at 37°C for 30 min, RA extract at a concentration of 49.6 mg prevented maximally LDH leakage (90.9%) and haemoglobin release (83.3%) in comparison to control having no RA extract (Fig. 5). Acetyl salicylate at a concentration of 1 mg prevented LDH leakage (51.5%) and haemoglobin release (41.75%) in comparison to control having no drug (Fig. 5). So, LDH leakage

measurements are also sensitive in monitoring the RBC membrane integrity and it has an added advantage that the coloured herbal extracts which will overshadow the haemoglobin colour when measured at 560 nm will have no interference in LDH activity measurements. Interestingly, membrane stabilization values for RA formula and acetyl salicylate at the highest concentration on the basis of haemoglobin measurements (Fig. 3) under experimental condition of hypotonic and heat combined stress which is more sensitive[29] are more closer to LDH leakage prevention values. From all these studies, it can be inferred that RA formula, its components except *Cydonia oblongata* and non-steroidal anti-inflammatory drug acetyl salicylate were able to stabilize sheep RBC membrane against osmotic and heat combined stress. Since the effects on RBC membranes can be extrapolated to lysosomal membrane system[7], the anti-inflammatory activity reported for certain herbal recipes viz RA formula, its components[16] and AR formula (unpublished) may be evoked through mechanism of lysosomal membrane stabilization. The components of AR formula except *Cydonia oblongata* may be also able to stabilize lysosomal membrane system.

SUMMARY

Hypotonicity and heat induced sheep erythrocytes lysis was chosen to study the membrane stabilizing activity of RA formula, its ocmponents viz. *Withania somnifera, Pyrethrum indicum, Merendra persica* and *Alpinia galanga* and AR formula and its components viz. *Ziziphus vulgaris, Cordia latifolia* and *Cydonia oblongata* and acetyl salicylate and diphenhydramine hydrochloride, an anti-allergic compound. When fresh sheep RBC (SRBC) was used, isosaline extracts of RA formula, AR formula, diphenhydramine hydrochloride and acetyl salicylate showed a biphasic response in membrane stabilization i.e. higher concentrations

caused the lysis of membranes. A similar response was seen in case of AR formula and its components when unfresh SRBC was used. Better dose dependent responses were observed for RA formula, its components and acetyl salicylate when unfresh SRBC system was employed. RA formula and acetyl salicylate prevented LDH leakage from SRBC. It has been proposed that these drugs exert anti-inflammatory activity possibly by stabilization of lysosomal membrane system.

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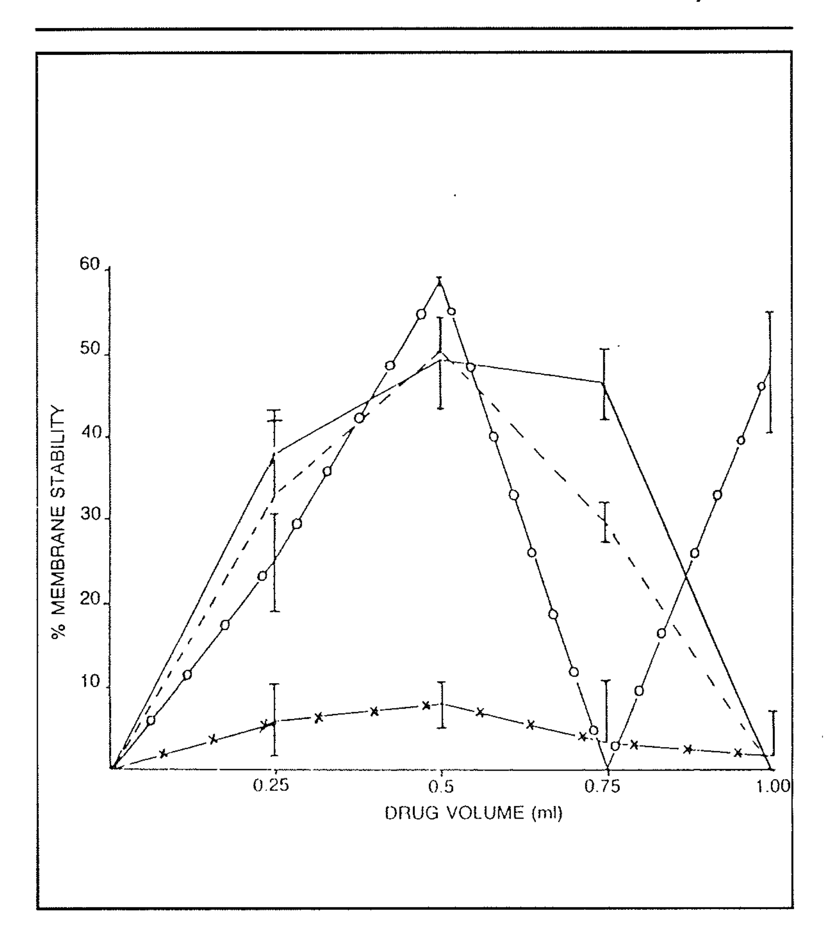


Fig. 1: The effect of isosaline extracts of certain herbal formulae on membrane stabilization in fresh SRBC subjected to hypotonic and heat stresses. 0.25% NaCl and 0.15 M phosphate buffer, pH 7.4 were used.

o———o	**AR formula**	**(25 mg/ml)**
- - - - - - - - - -	**RA formula**	**(49.8 mg/ml)**
————	**Diphenhydramine hydrochloride**	**(1 mg/ml)**
x———x	**Acetyl salicylate**	**(1 mg/ml)**

The values represent the mean of triplicates ± s.e.m.

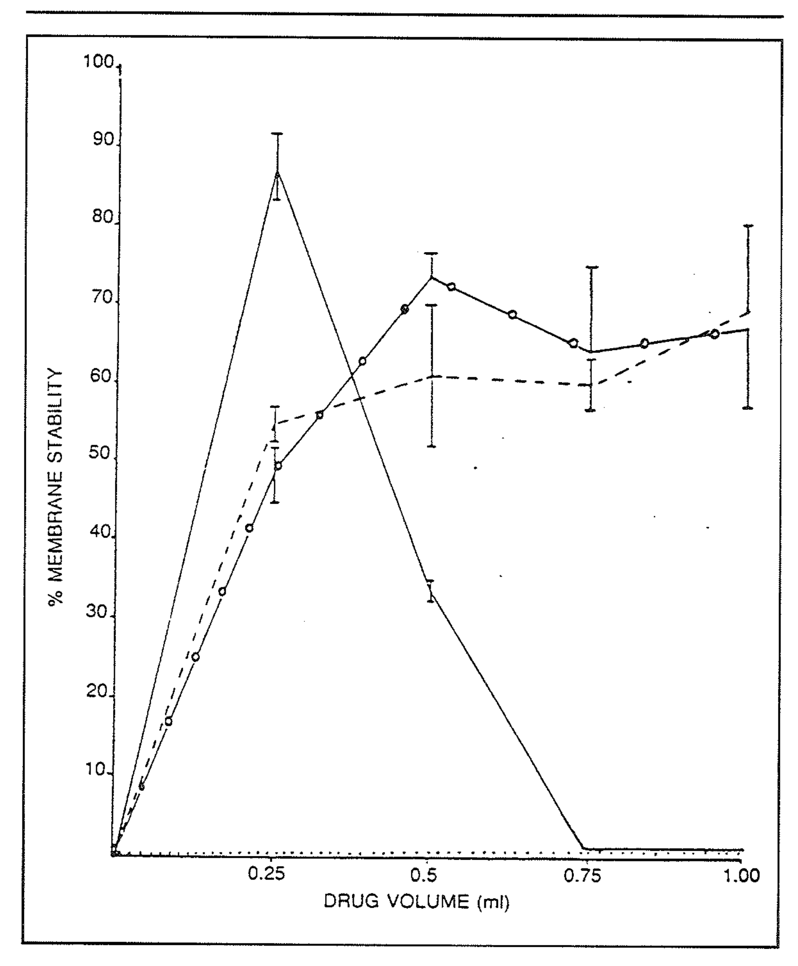


Fig. 2: Membrane stabilizing effect of AR formula and its components on unfresh SRBC subjected to hypotonic and heat streses. 0.25% NaCl and 0.15 M phosphate buffer, pH 7.4 wer eused.

————————	**AR formula**	**(49.8 mg/ml)**
o————o	***Cordia latifolia***	**(49.8 mg/ml)**
- - - - - - - - - - -	***Zizyphus vulgaris***	**(49.8 mg/ml)**
●●●●●●●●●●●●	***Cydonia oblongata***	**(49.8 mg/ml)**

The values represent the mean of tirplicates ± s.e.m.

Fig. 3: Membrance stabilizing effect of RA formula, its components, and acetyl salicylate on unfresh SRBC subjected to hypotonic and heat stresses. 0.36% NaCl and 0.15M phosphate buffer, pH 7.4 were used.

x———x	**RA formula**	**(49.8 mg/ml)**
————	***Pyrethrum indicum***	**(49.8 mg/ml)**
- - - - - - - - - -	***Withania somnifera***	**(49.8 mg/ml)**
o———o	***Merendra persica***	**(49.54 mg/ml)**
●●●●●●●●●●	***Alpinia galanga***	**(48.64 mg/ml)**
●●-●●-●●-●●-●	**Acetyl salicylate**	**(1 mg/ml)**

The values represent the mean of triplicates ± s.e.m.

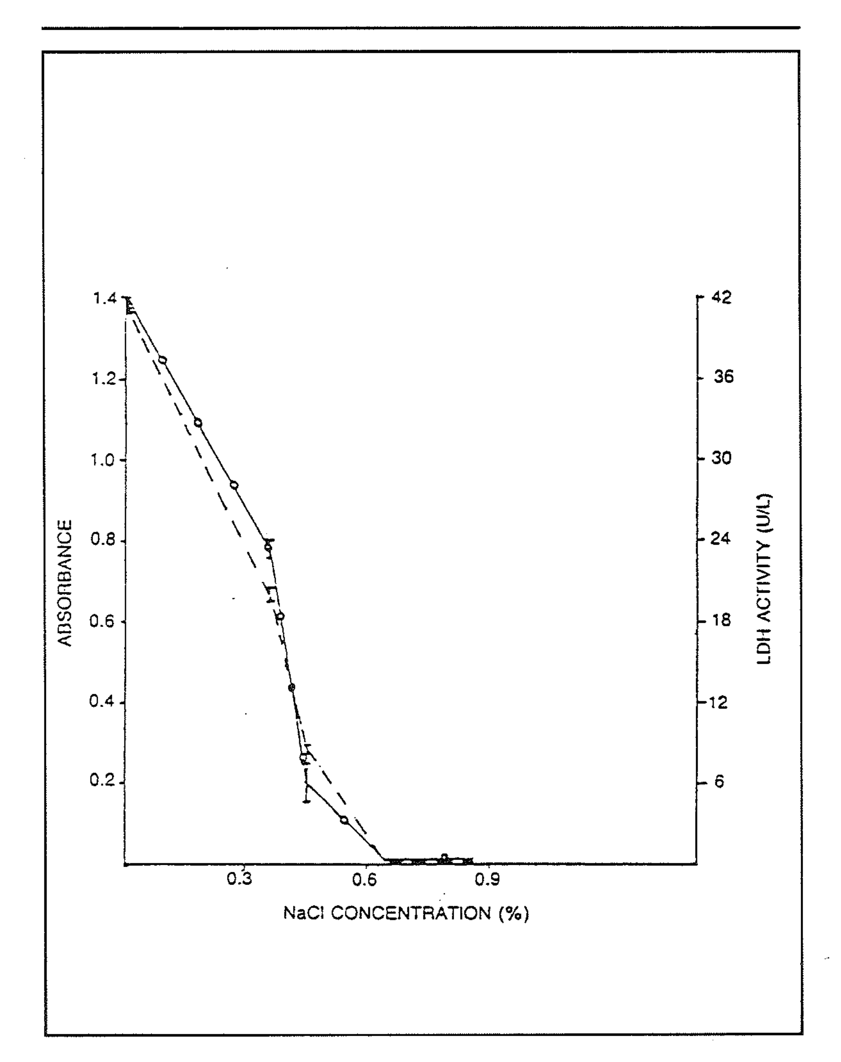


Fig. 4: The effect of different concentrations of NaCl solution on the release of haemoglobin and LDH from unfresh SRBC incubated at37°C for 30 min.

o———o LDH activity

----------- Haemoglobin absorbance measured at 560 nm.

The values represent the mean of triplicates ± s.e.m.

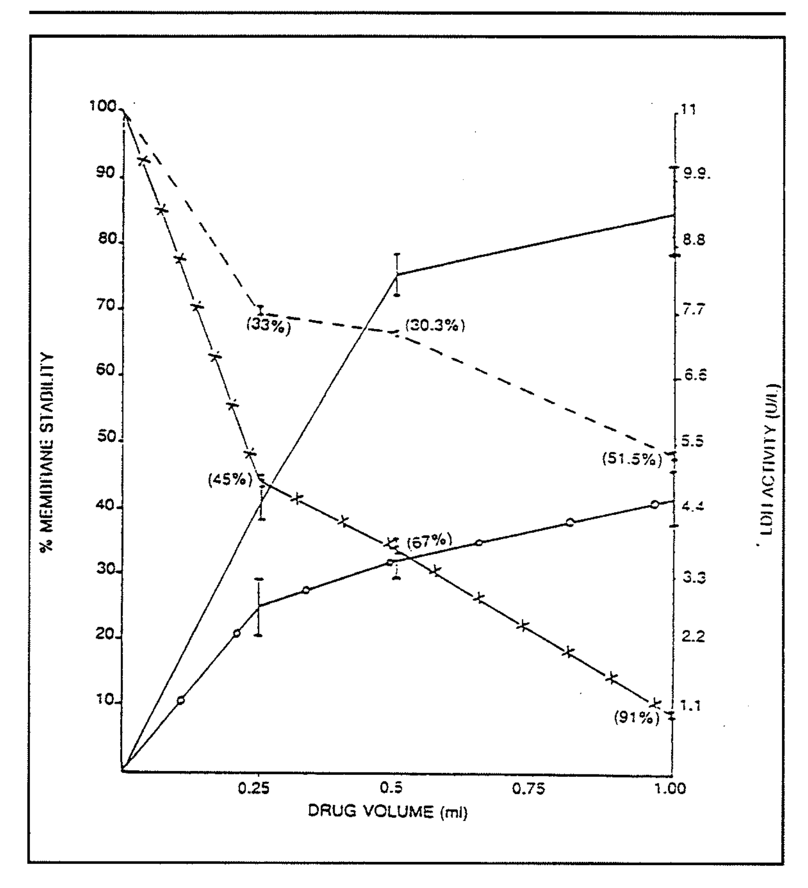


Fig. 5: The effect of RA formula and acetyl salicylate on the release of haemoglobin and LDH from unfresh SRBC subjected to hypotonic stress at 37°C.

———————— % membrane stability for RA formula (49.6 mg/ml) on the basis of Hb release

o———————o % membrane stability for acetyl salicylate (1 mg/ml) on the basis of Hb release

LDH activity (U/L) released in presence of different concentrations of RA extract.

- - - - - - - - - - LDH activity (U/L) released in presence of different concentrations of acetyl salicylate.

The values in parenthesis indicate percentage prevention of LDH leakage by RA extract and acetyl salicylate.

The value represent the mean of tirplicates ± s.e.m.

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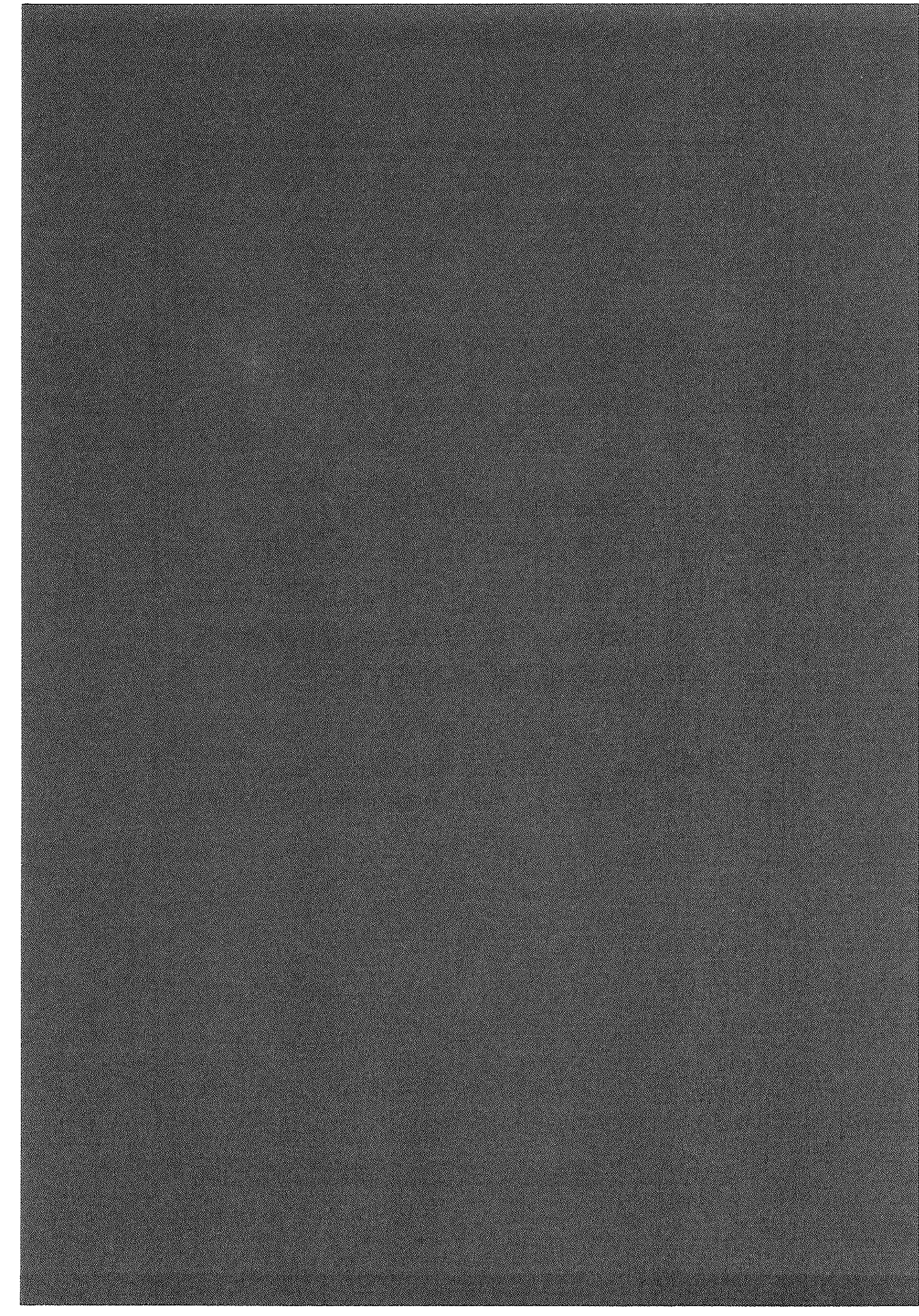
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SUPPRESSION OF CHOLESTEROGENESIS AND REDUCTION OF LDL CHOLESTEROL BY NATURAL PRODUCTS IN AVIAN AND MAMMALIAN SYSTEMS

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U.S.A.



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INTRODUCTION

Coronary heart disease is a major cause of death in the United States. Diets high in saturated fat and cholesterol raise the serum cholesterol level which is responsible for the elevation of serum lipoproteins, especially the *beta* or low density lipoproteins (Chol-LDL), which induce atherosclerosis. The effects of diet on plasma and liver cholesterol have been a subject of great interest in biomedical research. Several reports have suggested that the consumption of certain cereals has a cholesterol lowering effect on laboratory animals and humans[1]. Evidence implicating specific dietary components in this effect is contradictory, but plant proteins[2], fiber and lipids[3] have been suggested in various studies. Recently, it has been reported that a coarse oat fraction in conjunction with a high carbohydrate diet lowered serum total cholesterol and Chol-LDL and elevated high density lipoprotein (Chol-HDL) levels in several hypercholesterolemic humans[4]. In all these studies, the importance of essential microcomponents of plant origin in producing the hypocholesterolemic responses in humans had not been considered.

Our studies indicate that secondary products of plant metabolism arising from the mevalonate pathway have roles in the multivalent suppression of cholesterol biosynthesis. Mevalonate,

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the product of β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase is a key intermediate in pathways yielding steroids, isopentenyl tRNA and a variety of isoprenoid compounds, all of which are essential for the cell's growth and survival. A number of the latter play key roles in regualting plant germination, growth, maturation and senescence. These secondary products of plant metabolism are not degraded but instead remain in a storage form in seeds, bulbs and roots. Barley is a major dietary component of humans in the Mideastern part of the world where cardiovascular disease is quite rare. With this in mind, we examined chickens which has been fed diets supplemented with small grain (seeds), bulbs (garlic) or roots (ginseng) for alterations in lipid and cholesterol metabolism. Barley, oats, wheat, and rye diets produced inhibition of cholesterogenesis compared to a control diet[5].

The cholesterol inhibitor(s) appear to be distributed throughout the barley kernel as well as in the nonpolar and polar fractions of garlic and ginseng. The highest concentrations of cholesterol inhibitors in barley were found in the aleurone and subaleurone layers of endosperm (high-protein barley fraction, HPBF). Inclusions of 20% HPBF in the corn-based chicken diets caused a 16% increase in weight gain, an effect which has not been seen with barley or with HPBF when used as the main source (70-80%) of dietary protein[6]. This was accompanied by significant decreases in serum total cholesterol and Chol-LDL levels, without affecting the Chol-HDL[6].

This article is a summary of our research in which we have identified factors from three plant materials which suppress cholesterol biosynthesis. The isolation and the structural identification of the cholesterol inhibitors (I) and (II) and other components from the nonpolar fraction of HPBF will be described in detail with reference to *in vivo* and *in vitro* studies. These safe active agents present in barley, and possibly, garlic, ginseng, and oats suggest the

potential of using these plant materials as dietary supplements to lower Chol-LDL in humans suffering from hypercholesterolemia. The various effects of these components on lipid metabolism presented here have several implications for human nutrition and for possible control of cardiovascular disease in which Chol-LDL plays a key role.

MATERIALS AND METHODS

The protocols of our studies[5-13] examined the influence of barley and other grains [5-10], of barley milling fractions[10], of high-protein barley flour (HPBF) and its serial solvent extracts[6], of garlic and its serial solvent extracts[11,12] and of ginseng root and its serial solvent extracts[13] on avian hepatic cholesterol and lipid metabolism. Male and female chicks 1 day to 9 weeks of age and of broiler or layer strains were fed standard corn-soy diets or diets modified with the addition of test material for periods in excess of 19 days. The birds were housed in batteries under continuous illumination and were given free access to feed and water.

Procedures for the solvent fractionation of HPBF, garlic bulbs and ginseng root have been described[6, 11-13]. Briefly, the procedures involved successive extraction (repeated three times) with petroleum ether, ethyl acetate, methanol and water. The dried solvent fractions and the residue were added to the diet in proportion to the equivalent amount of 20% of the starting product.

The preparation of tissues for assays of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, cholesterol 7α-hydroxylase and fatty acid synthetase (FAS), the enzymic procedures and sources of chemicals as well as procedures for the estimations of plasma lipids and lipoproteins are given in reference 14.

A second protocol examined the influence of the solvent extracts on cholesterol and lipid metabolism by avian hepatocytes.

The preparation of the hepatocytes and the conditions of the incubation are described in reference 14.

RESULTS

Cereal grains in the diets of human populations are believed to be responsible for lowering serum total cholesterol levels by unknown mechanisms[15-18]. Our original dietary comparisons were of the effects of wheat, rye, oats and barley on cholesterol metabolism in chickens[7-10]. The stimuli for further studies described herein were the observations that isonitrogenous diets containing these cereals (Table 1) effectively lowered both plasma cholesterol levels and hepatic cholesterol synthesis. Hepatic lipogenesis, on the other hand, was enhanced by each of the cereals[8]. Barley has the greatest influence on these activities, reducing HMG-CoA reductase by 79% and plasma cholesterol by 45% and increasing FAS 5-fold as shown in Table 2. This observation prompted us to determine whether or not the active agents were confined to a specific portion of the barley kernel.

Commercial pearling of barley produces three fractions which are not sharply defined in terms of composition: (i) barley pearlings consisting of hull and bran; (ii) a high-protein barley flour (HPBF), consisting of aleurone and subaleurone layers of endosperm, and separable from the pearlings by sieving, and (iii) pearled barley consisting of starchy endosperm. Each fraction was fed in place of corn. The results indicated (Table 3) that the HPBF fraction most effectively lowered cholesterol synthesis to a level of 40% of that observed in controls. Lipogenesis and serum triglyceride levels were increased. Feed performance by chickens fed barley pearlings or HPBF was markedly depressed in these 3-week-old chickens.

The characterization of the constituents responsible for these effects began with the serial extraction of HPBF with solvents of increasing polarity[6]. The dried solvent fractions accounted for

18.8% of the HPBF (petroleum ether - 3.5%, ethyl acetate - 2.5%, methanol - 4.2% and water - 8.6%) and the residue, 81.2%. These fractions were fed at levels equivalent to 20% HPBF. Chicks fed with the petroleum ether solubles showed a 21% increase in weight gain which appeared to be related to a 40% decrease in FAS activity (Table 4). The constituents responsible for the suppression of HMG-CoA reductase and cholesterol 7 α-hydroxylase activities and the lowering of serum cholesterol levels were not segregated into a single solvent fraction (Table 4). An important finding was that the lowering of serum cholesterol was limited to that portion transported in the low density lipoprotein (LDL) fraction.

These results led us to examine other plant materials which are widely reported to have cholesterol-suppressive actions. Garlic paste (3.8 g) prepared from 5g garlic bulbs and its equivalent serial solvent fractions were fed at the levels shown on Table 5. All treatments, with the exception of the residue, suppressed HMG-CoA reductase, cholesterol 7α-hydroxylase and FAS and other lipogenic enzyme[12] activities in dose-dependent fashion[11]. The lowering of serum cholesterol was restricted to that in the LDL fraction (Table 5).

A brief report of the effects of Wisconsin ginseng root on human cholesterol metabolism[19] prompted us to feed chicks diets containing 0.25% ginseng root or its equivalent in serial solvent extracts[13]. Wisconsin or Chinese red ginseng root (0.25%) was added to the corn-based diet. Serum total cholesterol and Chol-LDL fell by at least 20% and 24% respectively and the Chol-HDL fell by 15%. Suppression of HMG-CoA reductase and cholesterol-7α-hydroxylase fell in the ranges recorded with 20% HPBF and 3.8% garlic paste as shown in Table 6. Serial solvent extraction revealed again that the effects were not clearly defined in terms of polarity. The petroleum ether soluble fraction of HPBF, garlic and

ginseng suppressed FAS and other lipogenic enzymes. However, the residue of ginseng, like that of garlic, elicited little response[13].

The polar serial solvent extracts of garlic also inhibited HMG-CoA reductase and FAS activities in avian isolated hepatocytes. The inhibitory action was dependent both on concentration (up to 3000 μg /ml) and duration of exposure (to 60 min) of either the methanol solubles or the water solubles (Tables 7 and 8). The time dependence was linear to 20 min, and the concentration dependence, to 100 μg/ml. HMG-CoA reductase and FAS activities under these assay conditions were 50% and 70% respectively of the control activities[11]. Similar results have been obtained using the methanol or water solubles of ginseng root (Table 9).

As mentioned above, when nonpolar (petroleum ether) and polar (methanol) soluble fractions of HPBF were fed to chickens, there was suppression of the hepatic activities of HMG-CoA reductase and cholesterol 7α-hydroxylase. The FAS was suppressed with nonpolar but stimulated by the polar soluble fraction[6]. The similar results for HMG-CoA reductase and FAS were duplicated with these fractions using isolated hepatocytes of rat and chicken (Tables 9, 10). This *in vitro* assay served as an inexpensive and rapid means of following the various active components as they were purified by high pressure liquid chromatography (HPLC) procedure (Fig. 1).

Different components of the nonpolar soluble fraction of HPBF were separated by semipreparative HPLC using an Ultrasphere C18 IP column (25 cm x 4.6 mm I.D., 5μ particle size), 50 μl of the sample was eluted with methanol at a flow rate of 1 ml per minute at 700 psi, using 200 nm as detecting wavelength. Each peak was scanned between 200-400 nm (Fig. 1) and tested in isolated hepatocytes of chicken using 200 μg/ml incubation and assayed for HMG-CoA reductase and FAS activities. Components of fractions 5 and 9 showed 68% and 59% inhibition of HMG-CoA reductase,

respectively. There was 38% induction with fraction 5 and 33% decrease with fraction 9 in the activity of FAS (Table 12A). These results were further confirmed by feeding these 10 components to chicks at levels reflecting their quantities in a 20% HPBF diet. HPLC fractions 5 and 9 (cholesterol inhibitors I and II, respectively) suppressed HMG-CoA reductase activity by 32% and 25% respectively. FAS activity in chicks fed fraction 5 was increased by 28% whereas fraction 9 decreased the activity by 26% (Table 12B).

The structure of cholesterol inhibitor I (fraction 5) isolated from nonpolar soluble fraction of HPBF was established as d-α-tocotrienol by high resolution mass spectrometry (Figure 2a). The mass measurement of the molecular ion peak (424^+) corresponded to a molecular formula of $C_{29}H_{44}O_2$. The fragmentation pattern and mass measurements of the different peaks indicated the presence of 6-chromanol nucleus with a methyl group ($C_{13}H_{17}O_2$) peak at 205^+ mass unit and 16-carbon unsaturated isoprenoid side chain at ring position 2. From the molecular ion peak there was a stepwise loss of 3 isoprenoid moieties of 69 mass units each time. First major loss from the molecular ion peak was 55 mass units of a C_4H_7 moiety, giving rise to an intense peak at 359^+. Further confirmation of the structure was obtained by the UV spectrum (Fig. 2b), consistent with the reported value of others which showed λmax at 292 nm, and closely related compounds such as Vitamin E and tocopherol series. The isolated cholesterol inhibitor I is very effective in inhibiting cholesterol biosynthesis both *in vivo* and *in vitro* using chicken hepatocytes at the level of 5-20 ppm in a number of repeated experiments (Table 13).

The structure of cholesterol inhibitor II (Fraction 9) was found to be 1, 3, dillinoleoyl, 2-linolenioylglycerol isolated from the nonpolar fraction of HPBF. The structure of this inhibitor was also established by its mass spectrum (Fig. 3) which sowed a characteristic fragmentation pattern of triglyceride with a molecular ion peak

at 876$^+$, corresponding to the molecular formula of $C_{57}H_{96}O_6$. The structure was confirmed by making p-bromophenacyl ester derivatives of the fatty acids obtained after saponification of the inhibitor II. The resulting p-bromo derivatives were identified by high pressure and recycling liquid chromatographic method using acetonitrile: water (90:10) as eluting solvent system on a C_{18}RP column against authentic compound derivatives of linoleic acid, α- and γ-linolenic acids using 254 nm as a detecting wavelength (Figs 4a and 4b). The mass spectra of these p-bromo derivatives showed, as expected, the double molecular ion peaks of bromine 79 and 81.

Cholesterol inhibitor II was found to be very effective in *in vivo* and *in vitro* studies (Table 14). Linoleic acid accounts for 55% of barley's total fatty acids. The presence of the gamma form of linolenic acid in this triglyceride appears to be one of the rare plant sourtes of this acid (cf. Evening Primrose oil), which is mainly found in animal systems. Apart from this triglyceride we were able to isolate a number of other isomers and its homologous series, but only this isomer was found to be effective in lowering cholesterol biosynthesis in chickens. A number of diglycerides have also been isolated from this fraction and identified as having different combinations of linoleic and α-or γ-linolenic acids.

Feeding trials with 21-day-old female rats showed a better growth rate with barley as compared to corn and a 50% reduction in HMG-CoA reductase activity. When compared to commercial chow diet, barley produced 20% less weight gain, but HMG-CoA reductase was reduced by 80%. Trials with 5-month-old Yorkshire and Hampshire gilts (82% corn or barley diets) produced about equal weight gains, but 19 to 25% reduction in HMG-CoA reductase in liver, adipose, intestine, lung and muslce tissues, and 17 to 18% less cholesterol in the plasma and muscle in the barley-fed animals (Tables 15, 16).

DISCUSSION

When the importance of cholesterol was first appreciated, a number of studies were carried out on the effects of various diets on blood cholesterol levels. Therefore, with all its limitations, plasma total cholesterol remains the best predictor of coronary heart disease risk in a human population. The predictive value of the total cholesterol may be improved slightly by consideration of the ratio between low density lipoproteins (Chol-LDL) and high density lipoproteins (Chol-HDL) cholesterol levels. Several reports covering the studies on human populations indicate that vegetarians and other individuals who consume diets principally of plant origin have lower risk factors for coronary heart disease, specifically, lower plasma cholesterol levels and lower ratios of plasma total cholesterol to high density lipoprotein (Chol-HDL) cholesterol[15-18].

A number of attempts to identify hypercholesterolemic factors in the Westernized diet and hypocholesterolemic factors in the vegetarian diet have been successful to varying degrees. Yet there continues to be considerable debate as to the specific factor. The most popular dietary practices recommended for the control of plasma cholesterol levels involve the selection of foods which decrease the quantity of cholesterol ingested and of dietary components which increase the excretion of cholesterol and its metabolites[20]. Both approaches increase endogenous cholesterol synthesis. This synthesis can be repressed by increasing cholesterol intake[21], an approach which maintains [22, 23] or elevates plasma cholesterol levels[24,25].

Recently, it was reported that the factors which elevate cholesterol are more potent when added to a purified diet[26,27]. This observation implies that crude diets contain factors which either 'detoxify' hypercholesterolemic factors or factors which act apart from cholesterol in suppressing cholesterol biosynthesis.

The results of the studies described here indicate that barley, garlic, ginseng and their nonpolar and polar soluble fractions contain minor components which suppress hepatic HMG-CoA reductase and lower plasma cholesterol level in chickens. HMG-CoA reductase is the ratelimiting step in cholesterol synthesis under most physiological conditions. The regulation of this enzyme is predominantly through changes in its mass via modulation of its synthesis and degradation[28,29], by hormones[30], steroids, oxygenated sterols[30], or by feedback[31]. Other controls may be exerted via its reversible phosphorylation[32], by cytosolic, noncatalytic proteins (y-and z-proteins;[33]) or by changes in membrane fluidity[34]. The suppression of HMG-CoA reductase by these plant constituents might be due to the increased cellular levels of products arising from the mevalonate pathway. According to Edwards et al.[29] these endogenous products destabilize HMG-CoA reductase resulting in an enhanced rate of its degradation. The plant materials examined here appear also to provide similar effectors of HMG-CoA reductase. Clegg et al.[35] have described the concentration dependent inhibition of HMG-CoA reductase activity following the *in vivo* administration of menthold to fed animals. Their studies ruled out all of the aforementioned modulations of HMG-CoA reductase activity except that imposed by reduction in enzyme mass.

The pure cholesterol inhibitors I and II from HPBF were found to be more potent than the crude extract. More significantly, these inhibitors were found to lower the serum total cholesterol levels and levels of Chol-LDL, without affecting the level of Chol-HDL. The suppression of lipogenesis and cholesterogenesis by the cholesterol inhibitor II may be due to the presence of γ-linolenic acid. A number of studies have strongly suggested that the cholesterol associated with LDL is preferentially taken up by intimal cells and is thus a positive risk factor in cardiovascular disease. Cholesterol associated with HDL is inversely related to cardiovascular disease

because HDL is involved in the removal of cholesterol from cells[36]. Although specific mechanism remains unknown, it is clear that dietary modifications influence HDL levels. The substitution of polyunsaturated for saturated dietary fat, for example, increases HDL cholesterol[37,38].

Another prevailing concept is that components of plant materials interfere with the reabsorption of bile acids therein causing a reduction in serum cholesterol levels[39-43]. Recent publications suggest that oat-bran[42] and whole grain[43] components exert hypocholesterolemic effect on the LDL fraction. Our data obtained by feeding solvent-extracted solids of HPBF, garlic and ginseng supplemented cornbased diets, on the other hand, point to a direct action of the plant material on cholesterol biosynthesis with a concomitant lowering of LDL-cholesterol.

The results presented herein are compatible with the observations of O'Brien and Reiser[26,27] namely that crude diets contain materials apart from cholesterol which suppress cholesterol biosynthesis. Therefore the present studies have demonstrated various dietary agents in food or natural products which can lower Chol-LDL levels in hypercholesterolemic subjects and thus prevent the onset of atherosclerosis in humans.

CONCLUSIONS

In the present studies we have shown that minor components of plant materials influence lipid and cholesterol metabolism. Chickens fed a diet containing barley or its milling products and other cereals or corn diet supplemented with garlic paste or ginseng root or their petroleum ether and methanol soluble fractions for 3 to 4 weeks, when compared to a corn-based diet (control) caused decreases between 40-60% in the serum total cholesterol level. This reduction was primarily in the cholesterol level of low density lipoprotein (Chol-LDL) fraction without affecting the cholesterol

concentrations in the high density lipoprotein (Chol-HDL). The hepatic β-hydroxy-β-methyglutaryl coezyme A (HMG-CoA) reductase and cholesterol 7α-hydroxylase (Chol-7α-hyd.) activities responded in parallel to these treatments.

Two hypocholesterolemic agents (cholesterol inhibitors I and II) have been isolated from the nonpolar fraction of high-protein barley flour. The structures of these cholesterol inhibitors were established as d-α-tocotrienol and 1,3-dilinoleoyl-2-linolenioylglycerol with the help of high resolution mass and UV absorption spectra. The purification of these inhibitors and other confirmatory characteristics were carried out by high pressure liquid chromatographic methods. These cholesterol inhibitors were found to be 400-700 times more effective than their respective crude fractions.

Despite the longstanding and widespread use of cereal grains, garlic and ginseng for food and feed, knowledge of their effects at the cellular levels is limited. Current interests in lipid metabolism, especially cholesterol, may serve as a stimulus for more definitive work on some of the minor constituents of the cereals, garlic and ginseng and their effects in humans. The present work with the isolation of pure cholesterol inhibitors I and II from barley demonstrates that there are many factors which come into play. Some of these may have therapeutic potential for those individuals who for genetic or purely dietary reasons are inclined toward abnormally high blood cholesterol concentrations.

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FOOTNOTE

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TABLE I: COMPOSITION OF ISONITROGENOUS CHICKEN DIETS

Ingredients	Diets[1]				
	A	B	C	D	E
A. Corn (9.1% protein)	61.5	-	-	-	-
B. Wheat (14.0% protein)	-	75.0	-	-	-
C. Barley (12.0% protein)	-	-	73.5	-	-
D. Oats (11.6% protein)	-	-	-	74.5	-
E. Rye (11.8% protein)	-	-	-	-	73.5
Soybean Meal (44% protein)	30.0	16.5	18.0	17.0	18.0
Meat Scrap	5.0	5.0	5.0	5.0	5.0
Alfalfa Meal (17% protein)	1.0	1.0	1.0	1.0	1.0

[1]All constituents used on an 'as is' basis. Grit (5%) was used at the expense of each diet. Also: dicalcium phosphate 1%; calcim carbonate 0.5%; mineral misture 0.5% consisting of (per kg) sodium chloride 2 mg, zinc sulfate 50 mg and manganese dioxide 50 mg, and vitamin mixture 0.5% consisting of (per kg) vitamin A 2,000 IU, vitamin D3,200 ICU, vitamin E 10 IU, vitamin KI 5 mg, choline 1.3 g, thiamin 1.8 mg, niacin 27 mg, riboflavin 3.6 mg, pyridozine 3 mg, calcium pantothenate 10 mg, vitamin B12 10 μg, and L-lysine-HCl 1 g, L-methionine 0.72 g.

TABLE 2: EFFECT OF CEREAL DIETS ON CHICK GROWTH AND LIPID METABOLISM[1]

Diet	Body Wt. g	Liver Wt. g	HMG-CoA Reductase[2]	Plasma Cholesterol mg/100 ml	FAS[3]
A. Corn	272 ± 7 (100)[4]	12.0 ± 0.8 (100)[4]	2.9 ± 0.6 (100)[4]	139 ± 7 (100)[4]	5.0 ± 0.5 (100)[4]
B. Wheat	221 ± 9 (81)	11.0 ± 1.2 (92)	2.2 ± 0.4 (76)	106 ± 4 (76)	11.2 ± 1.7 (224)
C. Barley	188 ± 6 (69)	8.2 ± 1.0 (68)	0.6 ± 0.1 (21)	76 ± 4 (55)	26.2 ± 2.4 (524)
D. Oats	195 ± 7 (72)	8.7 ± 1.4 (73)	1.2 ± 0.3 (41)	95 ± 4 (68)	7.7 ± 1.4 (154)
E. Rye	166 ± 9 (61)	6.8 ± 1.8 (57)	1.1 ± 0.2 (38)	105 ± 5 (76)	6.7 ±0.4 (134)

[1]Twenty-one day old chicks fed 18 days; N = 4 chickens/group; means ± SD; values in parentheses indicate percent of respective controls.

[2]n moles of mevalonic acid synthesized per min per g of liver.

[3]μ moles of NADPH oxidized per min per g of liver.

[4]Percentage of respective control activity data are in parentheses.

TABLE 3: EFFECT OF DIFFERENT FRACTIONS OF BARLEY KERNEL ON GROWHT AND LIPID METABOLISM IN FEMALE CHICKENS[1]

Diet	Body Wt. g	HMG-CoA Reductase[2]	Plasma Cholesterol mg/100ml	Plasma Triglycerides mg/100 ml	FAS[3]
Corn	263 ± 3.0^{a} (100)[4]	6.0 ± 1.0^{a} (100)[4]	165 ± 17^{a} (100)[4]	117 ± 7.0^{a} (100)[4]	4.8 ± 0.4^{a} (100)4
Barley	258 ± 4.0^{a} (98)	2.6 ± 0.8^{b} (43)	120 ± 14^{b} (73)	236 ± 15.0^{b,c} (202)	18.0 ± 0.3^{b} (375)
Barley Pearlings	116 ± 14.0^{b} (44)	3.5 ± 0.8^{b} (58)	126 ± 12^{b} (76)	229 ± 12.0^{b} (195)	17.6 ± 0.4^{b} (367)
HPBF	86 ± 4.0^{c} (33)	2.4 ± 0.6^{b} (40)	120 ± 11^{b} (73)	256 ± 11.0^{c} (219)	17.5 ± 0.2^{b} (364)
Pearled Barley	217 ± 6.0^{d} (83)	3.4 ± 0.4^{b} (51)	124 ± 15^{b} (75)	230 ± 19.0^{b} (197)	16.2 ± 0.3^{c} (338)

[1]Forty one-day old chickens were fed for 4 weeks; N = 8 chickens per group. Means ± S D; values in parentheses indicate percent of respective controls.a-d

[2]n moles mevalonic acid synthesized per min per g liver.

[3]μ moles NADPH oxidized per min per g liver.

[4]Percentage of respective control activity data are in parentheses.

[a-d]Means within a column and without a common superscript letter are significantly different; $P < 0.01$.

TABLE 4: EFFECT OF HPBF FRACTIONS ON HEPATIC ENZYME ACTIVITIES AND SERUM LIPIDS IN 12-WEEK-OLD CHICKENS[1]

Nutritional State	HMG-CoA Reductase[2]	Cholesterol 7α-Hydroxylase[3]	Fatty Acid Synthetase[4]	Concentration in Serum			
				Total Cholesterol	Triglycerides	Chol-HDL	Col-LDL
Corn	690 ± 44^{a} (100)[5]	6.7 ± 0.2^{a} (100)[5]	62 ± 8^{a} (100)[5]	208 ± 8^{a} (100)[5]	242 ± 18^{a} (100)[5]	68 ± 6^{a} (100)[5]	96 ± 9^{a} (100)[5]
Corn + 20% HPBF	460 ± 42^{a,b} (67)	3.7 ± 0.2^{b} (55)	72 ± 7^{a} (116)	178 ± 9^{b} (86)	308 ± 6^{b} (127)	66 ± 6^{a} (97)	76 ± 7^{b} (79)
Corn + Pet. Ether SF[6]	440 ± 37^{b} (64)	5.0 ± 0.4^{c} (75)	37 ± 3^{b} (60)	164 ± 7^{b} (79)	219 ± 4^{c} (91)	65 ± 4^{a} (96)	66 ± 7^{b} (69)
Corn + Ethyl Acetate SF[6]	460 ± 38^{a,b} (67)	5.5 ± 0.4^{c} (82)	113 ± 16^{c} (182)	204 ± 10^{a} (98)	231 ± 7^{a,c} (95)	67 ± 7^{a} (99)	88 ± 8^{a} (92)
Corn + Methanol SF[6]	350 ± 37^{c} (51)	3.4 ± 0.5^{b,d} (51)	121 ± 14^{c} (195)	159 ± 12^{b} (76)	314 ± 6^{b} (130)	62 ± 5^{a} (91)	42 ± 6^{c} (44)
Corn + Water SF[6]	370 ± 32^{c} (52)	4.1 ± 0.4^{d,e} (61)	151 ± 12^{d} (244)	202 ± 5^{a} (97)	262 ± 6^{a} (108)	64 ± 6^{a} (94)	94 ± 9^{a} (98)
Corn + Residue[6]	630 ± 43^{a} (92)	4.6 ± 0.5^{c,e} (69)	141 ± 11^{c,d} (227)	201 ± 6^{a} (97)	247 ± 7^{a} (102)	66 ± 4^{a} (97)	91 ± 7^{a} (95)

[1] Feeding period was three weeks; Time of killing was 0800; Data expressed as mean ± SD; N = 6 chickens per group.

[2] β-hydroxy-β-methylglutaryl-CoA reductase; $_{p}$moles of mevalonic acid synthesized per minute per mg of microsomal protein.

[3] pmoles of [^{14}C] cholesterol into [^{14}C] 7α-hydroxycholesterol per minute per mg of microsomal protein.

[4] nmoles of NA DPH oxidized per minute per mg of cytosolic protein.

[5] Percentage of respective control acticity data are in parentheses.

[6] SF = Soluble fraction equivalent to 20% HPBF in corn-based diet.

[a-e] Means within a column and without a common superscript letter are significantly different $P < 0.05$.

TABLE 5: EFFECT OF GARLIC AND ITS FRACTIONS ON HEPATIC ENZYME ACTIVITIES AND ON SERUM LIPIDS IN 12-WEEK-OLD WHL FEMALE CHICKENS[1]

Nutritional State	HMG-CoA Reductase[2]	Cholesterol 7α-Hydroxylase[3]	Fatty Acid Synthetase[4]	Concentration in Serum mg/100 ml			
				Total cholesterol	Triglycerides	Chol-HDL	Chol-LDL
Corn (Control)	909 $\pm 70^a$ (100)[5]	1.14 $\pm 0.08^a$ (100)[5]	175 $\pm 14.0^a$ (100)[5]	168.0 $\pm 2.0^a$ (100)[5]	125.1 $\pm 12.0^a$ (100)[5]	57.0 $\pm 4.0^a$ (100)[5]	86.0 $\pm 7.0^a$ (100)[5]
Corn + Garlic Paste	253 $\pm 40^b$ (28)	0.72 $\pm 0.04^b$ (63)	158 $\pm 12.0^{a,c}$ (90)	132.8 $\pm 10.0^b$ (79)	98.1 $\pm 10.0^b$ (78)	55.5 $\pm 8.0^a$ (97)	57.7 $\pm 5.0^b$ (67)
Corn + Garlic PESF	193 $\pm 30^c$ (21)	0.61 $\pm 0.03^c$ (54)	124 $\pm 11.0^b$ (71)	128.6 $\pm 8.0^b$ (76)	113.1 $\pm 10.0^a$ (90)	56.2 $\pm 8.0^a$ (99)	59.4 $\pm 4.0^b$ (69)
Corn + Garlic MESF	159 $\pm 30^c$ (17)	0.56 $\pm 0.04^c$ (49)	141 $\pm 11.0^c$ (81)	126.5 $\pm 4.0^b$ (75)	92.6 $\pm 11.0^b$ (74)	57.0 $\pm 5.0^a$ (100)	51.0 $\pm 4.0^b$ (59)
Corn + Galric WASF	192 $\pm 28^c$ (21)	0.65 $\pm 0.05^{b,c}$ (57)	145 $\pm 8.0^c$ (83)	134.7 $\pm 11.0^b$ (80)	96.3 $\pm 10.0^b$ (77)	54.7 $\pm 6.0^a$ (96)	60.7 $\pm 5.0^b$ (71)
Corn + Garlic Residue	781 $\pm 62^a$ (86)	0.93 $\pm 0.06^d$ (82)	172 $\pm 13.0^a$ (98)	152.2 $\pm 14.0^a$ (91)	121.7 $\pm 10.0^a$ (97)	53.5 $\pm 4.0^a$ (94)	74.4 $\pm 7.0^a$ (87)
Corn + Garlic Oil (commercial)	230 $\pm 30^b$ (26)	0.67 $\pm 0.05^c$ (59)	160 $\pm 4.0^a$ (91)	128.9 $\pm 7.0^b$ (77)	115.1 $\pm 7.0^a$ (92)	54.5 $\pm 3.0^a$ (96)	61.2 $\pm 6.0^b$ (71)

[1] Feeding period was four weeks; Time of killing was 0800; Data expressed as mean ± SD; N = 8 chickens per group; IMG-CoA reductase = β-hydroxy-β-methylglutaryl-CoA reductase. PESF, MESF, WASF = petroleum ether, methanol and water soluble fractions of garlic, respectively.

[2] $_p$moles of mevalonic acid synthesized per minute per mg. of microsomal fraction.

[3] $_p$moles of [^{14}C] cholesterol into [^{14}C] 7α-hydroxycholesterol per minute per mg. of microsomal fraction.

[4] nmoles of NA DH oxidized per minute per mg. of cytosolic fraction.

[5] Percentage of respective control activity data are in parentheses.

[a-d] Values not sharing a common superscript letter are different at $P < 0.01$.

TABLE 6: EFFECT OF GINSENG AND ITS FRACTIONS ON HEPATIC ENZYME ACTIVITIES AND SERUM LIPIDS IN 12-WEEK-OLD FEMALE (WLH) CHICKENS[1]

Nutritional State	HMG-CoA Reductase[2]	Cholesterol 7α -Hydroxylase[3]	Fatty Acid Synthetase[4]	Concentration in Serum mg/100 ml		
				Total Choelsterol	Chol-HDL	Chol-LDL
Corn (control)	909 ± 72^{a} (100)[5]	1.14 ± 0.04^{a} (100)[5]	145 ± 4^{a} (100)[5]	167.9 ± 10^{a} (100)[5]	61.4 ± 5^{a} (100)[5]	86.0 ± 10^{a} (100)[5]
Corn + Ginseng Powder	295 ± 27^{b} (32)	0.71 ± 0.02^{b} (62)	141 ± 7^{a} (97)	134.9 ± 6^{b} (80)	51.6 ± 7^{a} (84)	65.4 ± 6^{b} (76)
Corn + Ginseng PESF	284 ± 17^{b} (31)	0.53 ± 0.01^{c} (46)	107 ± 7^{b} (74)	112.4 ± 6^{c} (67)	52.2 ± 7^{a} (85)	46.2 ± 6^{c} (54)
Corn ± Ginseng MESF	332 ± 30^{b} (37)	0.64 ± 0.02^{d} (56)	141 ± 9^{a} (97)	140.4 ± 8^{b} (84)	52.0 ± 5^{a} (85)	69.5 ± 5^{b} (81)
Corn + Ginseng WASF	307 ± 26^{b} (34)	0.56 ± 0.02^{d} (50)	125 ± 6^{c} (86)	128.9 ± 10^{b,c} (77)	56.9 ± 4^{a} (93)	47.6 ± 6^{c} (55)
Corn + Ginseng Residue	820 ± 42^{a} (90)	1.13 ± 0.02^{a} (99)	144 ± 5^{a} (99)	163.4 ± 7^{a} (98)	59.7 ± 5^{a} (97)	83.9 ± 7^{a} (98)

[1]Feeding period was four weeks; Time of killing was 0800; Data expressed as mean ± SD; N = 8 chickens per group; HMG-CoA reductase = β-hydroxy-β-methylglutaryl-CoA reductase. PESF, MESF, and WASF = petroleum ether, methanol and water soluble fractions of ginseng, respectively.

[2] $_{p}$moles of mevalonic acid synthesized per minute per mg. of microsomal protein.

[3] $_{p}$moles of [^{14}C] cholesterol into [^{14}C] 7α-hydroxycholesterol per minute per mg. of microsomal protein.

[4] nmoles of NADPH oxidized per minute per mg. of cytosolic protein.

[5] Percentage of respective control activity data are in parentheses.

[a-d] Values not sharing a common superscript letter are different at $P < 0.01$.

TABLE 7: EFFECT OF DIFFERENT CONCENTRATIONS OF METHANOL AND WATER SOLUBLE FRACTIONS OF GARLIC ON THE ENZYMIC ACTIVITIES OF β-HYDROXY-β-METHYLGLUTARYL-CoA REDUCTASE AND FATTY ACID SYNTHETASE IN ISOLATED HEPATOCYTES OF FEMALE CHICKENS[1]

Methanol or water soluble fractions of galric	β-Hydroxy-β-methylglutaryl-CoA reductase[2]		Fatty acid synthetase[3]	
Concentration in μg/ml	Methanol Soluble fraction	Water soluble fraction	Methanol soluble fraction	Water soluble fraction
0.0	22.5 (100)[4]	24.3 (100)[4]	69.9 (100)[4]	77.8 (100)[4]
25.0	15.3 (68)	14.5 (60)	68.8 (98)	75.2 (97)
50.0	14.7 (65)	14.0 (58)	65.2 (93)	71.3 (92)
75.0	11.2 (50)	10.2 (42)	59.7 (85)	65.4 (84)
100.0	10.4 (46)	9.5 (39)	53.8 (77)	61.2 (79)
200.0	9.7 (43)	9.0 (37)	46.7 (67)	54.3 (70)
300.0	8.6 (38)	9.1 (37)	44.4 (64)	52.2 (67)

[1] Eight-week-old female chickens were fed standard corn-soybean diets. They were fasted for 48 hr and refed 72 hr prior to the preparation of liver perfusion. Incubation period was 15 minutes. Values represent means of replicate within incubation set.

[2] $_p$moles of mevalonic acid synthesized/minute/mg of microsomal fraction.

[3] nmoles of NADPH oxidized/minute/mg of cytosolic fraction.

[4] Percentage of respective control activity data are in parentheses.

TABLE 8: EFFECT OF LENGTH OF INCUBATION WITH METHANOL AND WATER SOLUBLE FRACTIONS OF GARLIC ON THE ENZYME ACTIVITIES OF β-HYDROXY-β-METHYLGLUTARYL-CoA REDUCTASE AND FATTY ACID SYNTHETASE IN ISOLATED HEPATOCYTES OF MALE CHICKENS[1]

Incubation Time min	β-Hydroxy-β-methylglutaryl-CoA reductase[2]		Fatty acid synthetase[3]	
	Methanol soluble fraction	Water soluble fraction	Methanol soluble fraction	Water soluble fraction
0	15.5 (100)[4]	18.5 (100)[4]	52.0 (100)[4]	58.5 (100)[4]
5	13.0 (84)	14.5 (78)	46.0 (88)	52.5 (90)
10	11.5 (74)	11.0 (59)	44.0 (85)	46.0 (79)
15	9.5 (61)	10.0 (54)	40.5 (78)	43.5 (74)
20	8.5 (55)	9.5 (51)	38.4 (73)	41.0 (70)
40	8.0 (52)	9.0 (49)	37.0 (71)	41.0 (70)
60	8.0 (52)	9.0 (49)	35.5 (68)	37.0 (63)

[1]Eight-week-old male chickens were fed standard corn-soybean diets. They were fasted for 48 hrs and refed 72 hrs prior to the preparation of liver perfusion. Each incubation contains 100 μg of methanol or water soluble fractions of garlic; value represents means of replicate within incubation set.

[2] $_p$moles of mevalonic acid synthesized/minute/mg of microsomal fraction.

[3] $_n$omoles of NADPH oxidized/minute/mg of cytosolic fraction.

[4] Percentage of respective control activity data are in parentheses.

TABLE 9: EFFECT OF DIFFERENT CONCENTRATIONS OF METHANOL AND WATER SOLUBLE FRACTIONS OF GINSENG ON THE ENZYMIC ACTIVITIES OF β-HYDROXY-β-METHYLGLUTARYL-CoA REDUCTASE AND FATTY ACID SYNTHETASE IN ISOLATED HEPATOCYTES OF FEMALE CHICKENS[1]

Methanol or Water soluble fractions of Ginseng	β-Hydroxy-β-methylglutaryl-CoA reductase[2]		Fatty acid synthetase[3]	
Concentration in μg/ml	Methanol soluble fraction	Water soluble fraction	Methanol soluble fraction	Water soluble fraction
0.0	24.3 (100)[4]	21.7 (100)[4]	48.8 (100)[4]	56.2 (100)[4]
25.0	18.9 (78)	20.8 (96)	44.2 (91)	53.4 (95)
50.0	17.4 (72)	18.6 (86)	40.6 (83)	47.3 (84)
75.0	16.1 (66)	17.5 (81)	37.6 (77)	45.2 (80)
100.0	15.2 (63)	17.1 (79)	35.2 (72)	40.7 (72)
200.0	14.8 (61)	16.8 (77)	34.6 (72)	39.2 (70)
300.0	14.6 (60)	16.7 (77)	34.8 (71)	39.0 (69)

[1]Seven-week-old female chickens were fed standard corn-soyben diets. They were fasted for 48 hrs and refed 72 hrs prior to the preparation of liver perfusion. Incubation period was 15 minutes. Values represent means of replicate within incubations set.

[2] $_p$moles of mevalonic acid synthesized/minute/mg of microsomal fraction.

[3] $_n$moles of NADPH oxidized/minute/mg of cytosolic fraction.

[4] Percentage of respective control activity data are in parentheses.

TABLE 10: EFFECT OF DIFFERENT CONCENTRATIONS OF PETROLEUM-ETHER SOLUBLE FRACTION (PESF) OF HPBF ON THE ENZYMIC ACTIVITIES OF β-HYDROXY-β-METHYLGLUTARYL-CoA REDUCTASE AND FATTY ACID SYNTHETASE IN ISOLATED HEPATOCYTES OF CHICKEN AND RAT[1]

PESF of HPBF Concentration (mg/ml)	β-Hydroxy-β-Methylglutaryl-CoA Reductase[2]				Fatty Acid Synthetase[3]			
	Chicken		Rat		Chicken		Rat	
0	19.5	(100)[4]	17.5	(100)[4]	17.6	(100)[4]	17.2	(100)[4]
1	18.0	(92)	16.1	(92)	15.8	(90)	16.2	(98)
2	15.9	(82)	14.7	(84)	14.8	(84)	15.3	(89)
4	15.0	(77)	14.2	(81)	12.8	(73)	14.2	(83)
6	14.1	(72)	13.7	(78)	12.6	(72)	13.3	(77)
8	13.2	(68)	13.5	(77)	11.2	(64)	12.2	(71)
10	12.9	(66)	13.0	(74)	10.3	(59)	11.5	(67)

[1] Eight-week-old female chickens and six-week-old male Sprague-Dawley rats were fed standard corn-soy and Purina chow diets. They were fasted for 48 hrs and refed 72 hrs prior to the preparation of liver perfusion. Incubation period was 15 minutes. HPBF = high protein barley flour.

[2] $_p$moles of mevalonic acid synthesized per minute per mg of microsomal fraction.

[3] $_n$moles of NADPH oxidized per minute per mg of cytosolic fraction.

[4] Percentage of respective control activity data are in parentheses.

TABLE 11: EFFECT OF DIFFERENT CONCETRATIONS OF METHANOL SOLUBLE FRACTION (MESF) OF HPBF ON THE ENZYMIC ACTIVITIES OF β-HYDROXY-β-METHYLGLUTARYL-CoA REDUCTASE AND FATTY ACID SYNTHETASE IN ISOLATED HEPATOCYTES OF CHICKEN AND RAT[1]

MESF of HPBF Concentration (mg/ml)	β-Hydroxy-β-Methylglutaryl-CoA Reductase[2]		Fatty Acid Synthetase[3]	
	Chicken	Rat	Chicken	Rat
0	20.0 (100)[4]	19.5 (100)[4]	21.7 (100)[4]	19.8 (100)[4]
1	17.5 (88)	18.4 (94)	23.9 (110)	20.8 (105)
2	15.5 (78)	17.6 (90)	25.4 (117)	21.9 (111)
4	12.5 (63)	17.2 (88)	26.6 (123)	22.8 (115)
6	10.0 (50)	15.0 (77)	27.7 (128)	23.9 (121)
8	8.5 (43)	14.8 (76)	28.2 (130)	26.8 (135)
10	8.0 (40)	14.5 (74)	29.9 (138)	28.6 (144)

[1] Eight-week-old female chickens and six-week-old male Sprague-Dawley rats were fed standard corn-soy and Purina chow diets. They were fasted for 48 hrs and refed 72 hrs prior to the preparation of liver perfusion. Incubation period was 15 mintues. HPBF = high protein barley flour.

[2] pmoles of mevalonic acid synthesized per minute per mg of microsomal fraction.

[3] $_n$moles of NADPH oxidized per minute per mg of cytosolic fraction.

[4] Percentage of respective contorl activity data are in parentheses.

TABLE 12: EFFECT OF DIFFERENT HPLC PURIFIED COMPOUNDS FROM PETROLEUM-ETHER SOLUBLE FRACTION OF HPBF ON THE ENZYMIC ACTIVITIES OF β-HYDROXY -β-METHYLGLUTARYL-CoA REDUCTASE AND FATTY ACID SYNTHETASE

(A) In Isolated Hepatocytes of chicken[1] *(In vitro)*.

HPLC Purified Components from PESF	Concentration (μg/ml)	β-Hydroxy-β-Methylglutaryl-CoA Reductase[2]	Fatty Acid Synthetase[3]
Control	(0)	75.9 (100)[4]	63 (100)[4]
1	200	74.2	69
2	200	78.4	67
3	200	86.8	71
4	200	64.9 (86)	72
5 •	200	24.6 (32)	87 (138)
6	200	74.0	61
7	200	68.9	57
8	200	70.8	62
9 •	200	31.3 (41)	42 (67)
10	200	73.7	65

(B) Three-week-Broiler Male Chickens[5] *(In vivo)*.

Nutritional State	B-Hydroxy-β-Methylglutaryl-CoA Reductase[2]	Fatty Acid Synthetase[3]
Corn (Control)	198 ± 15[5,a]	168 ± 20[5,a]
Corn + PESF of HPBF	142 ± 8[b]	146 ± 14[b]
Corn + HPLC Peak #1	193 ± 16[b]	178 ± 29[a]
Corn + HPLC Peak #2	188 ± 11[a]	166 ± 28[a]
Corn + HPLC Peak #3	194 ± 12[a]	188 ± 35[a]
Corn + HPLC Peak #4	186 ± 10[a]	177 ± 41[a]
Corn + HPLC Peak #5 ●	134 ± 6[c]	215 ± 18[c]
Corn + HPLC Peak #6	184 ± 17[a]	171 ± 27[a]
Corn + HPLC Peak #7	182 ± 9[a]	177 ± 29[a]
Corn ± HPLC Peak #8	189 ± 13[a]	164 ± 27[a]
Corn + HPLC Peak #9 ●	149 ± 8[b]	124 ± 19[d]
Corn + HPLC Peak #10	179 ± 12[a]	177 ± 28[a]

[1]Twelve-week-old female chickens were fed standard corn-soy diet. They were fasted for 48 hrs and refed 72 hrs prior to the preparation of liver perfusion. Incubation period was 15 minutes. HPLC = high pressure liquid chromatography; PESF = petroleum-ether soluble fraction; HPBF = high protein barley flour.

[2]$_p$moles of mevalonic acid synthesized per minute per mg of microsomal fraction.

[3]$_n$moles of NADPH oxidized per minute per mg of cytosolic fraction.

[4]Percentage of respective control activity data are in parentheses.

[5]Feeding period was three weeks; Time of killing was 0800 hrs; Data expressed as mean ± SD; N = 9;3-week-old broiler male chickens per group.

[a-d]Means within a line and without a common superscript are different at $p < 0.01$.

TABLE 13: EFFECT OF CHOLESTEROL INHIBITOR ISOLATED FROM PETROLEUM-ETHER SOLUBLE FRACTION OF HPBF ON THE ENZYMIC ACTIVITIES OF β-HYDROXY-β-METHYGLUTARYL-CoA REDUCTASE AND FATTY ACID SYNTHETASE.

Three-Week-Old Broiler Male Chickens[1] *(in vivo)*.

Nutritional State (Concentration in ppm)	β-Hydroxy-β-Methylglutaryl-CoA Reductase[2]	Fatty Acid Synthetase[3]
Corn (control)	198 ± 15[a] (100)[4]	168 ± 14[a] (100)[4]
Corn + Chol. Inhib. I 2.5	172 ± 12[a,b] (87)	190 ± 12[a] (118)
Corn + Chol. Inhib. I 5.0	161 ± 12[b] (81)	202 ± 10[b] (120)
Corn ± Chol. Inhib. I 10.0	144 ± 9[b] (73)	210 ± 15[b,c] (125)
Corn + Chol. Inhib. I 15.0	135 ± 8[c,b] (68)	218 ± 17[b,c] (130)
Corn + Chol. Inhib. I 20.0	130 ± 6[c,b] (66)	235 ± 18[c] (140)

In Isolated Hepatocytes of Chicken[5] *(in vitro)*.

Chol Inhib. I Concentration (μg /ml)[6]	β-Hydroxy-β-Methylglutaryl -CoA Reductase[2]	Fatty Acid Synthetase[3]
0	52 (100)[7]	72 (100)[7]
5	45 (87)	88 (122)
10	40 (77)	97 (135)
15	31 (60)	112 (156)
20	30 (57)	126 (175)
25	28 (54)	135 (188)
50	22 (42)	148 (188)
100	23 (44)	154 (214)

[1]Feeding period was three weeks; Time of killing was 0800; Data expressed as mean ± SD ; N = 9 chickens per group.

[2] pmoles of mevalonic acid synthesized per minute per mg of microsomal protein.

[3] nmoles of NADPH oxidized per minute per mg of cytosolic protein.

[4] Percentage of respective control activity data are in parentheses.

[a-c] Values not sharing a common superscript letter are different at $P < 0.01$.

[5] Ten-week-old female chickens were fed standard corn-soy diet. They were fasted for 48 hrs and refed 72 hrs prior to the preparation of liver perfusion.

[6] Incubation period was 15 minutes. Values represent means of two replicates within incubation set.

[7] Percentage of respective control activity data are in parentheses. The results presented above were carried out by using the cells from one liver.

TABLE 14: EFFECT OF CHOLESTEROL INHIBITOR II FROM PETROLEUM-ETHER SOLUBLE FRACTION OF HPBF ON THE ENZYMIC ACTIVITIES OF β-HYDROXY-β-METHYLGLUTARYL-CoA REDUCTASE AND FATTY ACID SYNTHETASE.

Thirteen-week-old WLH Male Chickens[1] *(in vivo)*

Nutritional State (Concentration in ppm)	β-Hydroxy-β-Methylglutaryl-CoA Reductase[2]	Fatty Acid Synthetase[3]
Corn (control)	484 ± 35[a] (100)[4]	262 ± 7[a] (100)[4]
Corn + Chol. Inhib. II 2.5	309 ± 24[b] (64)	255 ± 8[a,b] (97)
Corn + Chol. Inhib. II 5.0	295 ± 21[b,c] (61)	240 ± 7[b] (92)
Corn + Chol. Inhib. II 10.0	281 ± 20[b,c] (58)	235 ± 8[b,c] (90)
Corn + Chol. Inhib. II 15.0	278 ± 18[b,c] (57)	231 ± 6[b,c] (88)
Corn + Chol. Inhib. II 20.0	265 ± 16[c] (55)	225 ± 4[c] (86)

In Isolated Hepatocytes of chicken[5] *(in vitro)*

Chol. Inhib. II Concentration (μg/ml)[6]	β-Hydroxy-β-Methylglutaryl-CoA Reductase[2]	Fatty Acid Synthetase[3]
0	42 (100)[7]	46 (100)[7]
5	39 (93)	36 (78)
10	35 (83)	32 (70)
15	30 (71)	30 (65)
20	26 (62)	30 (65)
25	25 (60)	28 (61)
50	24 (57)	26 (57)
100	22 (52)	29 (63)

[1]Feeding period was three weeks; Time of killing was 0800; Data expressed as mean ± SD; N = 9 chickens per group.

[2] p-moles of mevalonic acid synthesized per minute per mg of microsomal protein.

[3] nmoles of NADPH oxidized per minute per mg of cytosolic protein.

[4] Percentage of respective control activity data are in parentheses.

[a-c] Values not sharing a common superscript letter are different at $P < 0.01$.

[5]Ten-week-old female chickens were fed standard corn-soy diet. They were fasted for 48 hrs and refed 72 hrs prior to the presentation of liver perfusion.

[6]Incubation period was 15 minutes. Values represent means of two replicates within incubation set.

[7]Percentage of respective control activity data are in parentheses. The results presented above were carried out by using the cells from one liver.

TABLE 15: EFFECT OF CEREALS ON THE ACTIVITIES OF β-HYDROXY-β-METHYLGLUTARYL-CoA REDUCTASE AND CHOLESTEROL 7α-HYDROXYLASE IN CERTAIN SWINE TISSUES[1]

Nutritional State	Tissue					
	Liver	Adipose (Inside)	Adipose (Outside)	Intestine	Lung	Muscle[4]
Corn	HMG[2] 163.0 ± 9.0^{a} (100)[3]	485.0 ± 10^{a} (100)[3]	478.2 ± 15.0^{a} (100)[3]	212.3 ± 9.0^{a} (100)[3]	78.3 ± 5.0^{a} (100)[3]	55.2 ± 2.0^{a} (100)[3]
	7α-OH[2] 4.6 ± 0.2^{a} (100)[3]	19.7 ± 1.0^{a} (100)[3]	11.8 ± 1.5^{a} (100)[3]	5.4 ± 0.5^{a} (100)[3]	1.8 ± 0.6^{a} (100)[3]	1.1 ± 0.1^{a} (100)[3]
Barley	HMG 142.0 ± 3.0^{b} (87)	395.0 ± 10^{b} (81)	381.2 ± 9.0^{b} (80)	158.2 ± 8.0^{b} (75)	62.1 ± 5.0^{b} (79)	42.3 ± 2.0^{b} (77)
	7α-OH 3.3 ± 0.2^{b} (72)	16.8 ± 1.0^{b} (85)	8.4 ± 0.4^{b} (71)	4.1 ± 0.3^{b} (76)	1.4 ± 0.2^{b} (78)	0.8 ± 0.1^{b} (73)

[1] Feeding period was three weeks. Time of killing was 0800 hours. Data expressed as means ± SD; N = 5 swine per group.

[2] HMG = β-hydroxy-β-methylglutaryl-CoA reductase, and 7αOH = cholesterol 7 α-hydroxylase as p-moles of mevalonic acid synthesized per min per mg of microsomal protein (HMG) or $_{p}$moles of [^{14}C] cholesterol into [^{14}C] 7α-hydroxy cholesterol per min per mg of microsomal protein (7αOH).

[3] Percentage of respective control (corn-diet) activity data are in parentheses.

[a-b] Means for a given enzyme within a column and without a common superscript letter are significanly different $p < 0.01$.

[4] Enzyme activities recorded for the muscle tissue may actually represent contribution of the intramuscular fat cells which account for about 5% of the weight of the semimembranous muscle.

TABLE 16: EFFECT OF CEREALS SUPPLEMENTED DIETS ON THE LEVEL OF CHOLESTEROL IN PLASMA AND MUSCLE OF SWINE[1]

Nutritional State	Days of feed (Plasma[2])					(Muscle[3])
	0	5	10	15	21	21
Corn	92.5 ± 2.0	98.3 ± 3.0[a] (100)[4]	96.7 ± 3.0a (100)[4]	97.3 ± 4.0[a] (100)[4]	95.3 ± 3.0[a] (100)[4]	83.0 ± 4.6[a]
Barley	98.6 ± 2.0	94.2 ± 3.0[b] (96)	87.6 ± 3.0[b] (89)	81.7 ±2.0[c] (84)	78.6 ± 2.0[c] (82)	69.2 ± 2.0[b] (83)

[1] Feeding period was three weeks. Time of killing was 0800 hours. Data expressed as means ± SD; N = 5 swine per groups.

[2] The cholesterol concentration is expressed as mg/100 ml of plasma.

[3] The cholesterol concentration is expressed as mg/100 gm of muscle.

[4] Percentage of respective control (corn-diet) concentration data are in parentheses.

[a-c] Means within a column and without a common small superscript letter are significantly different $p < 0.01$.

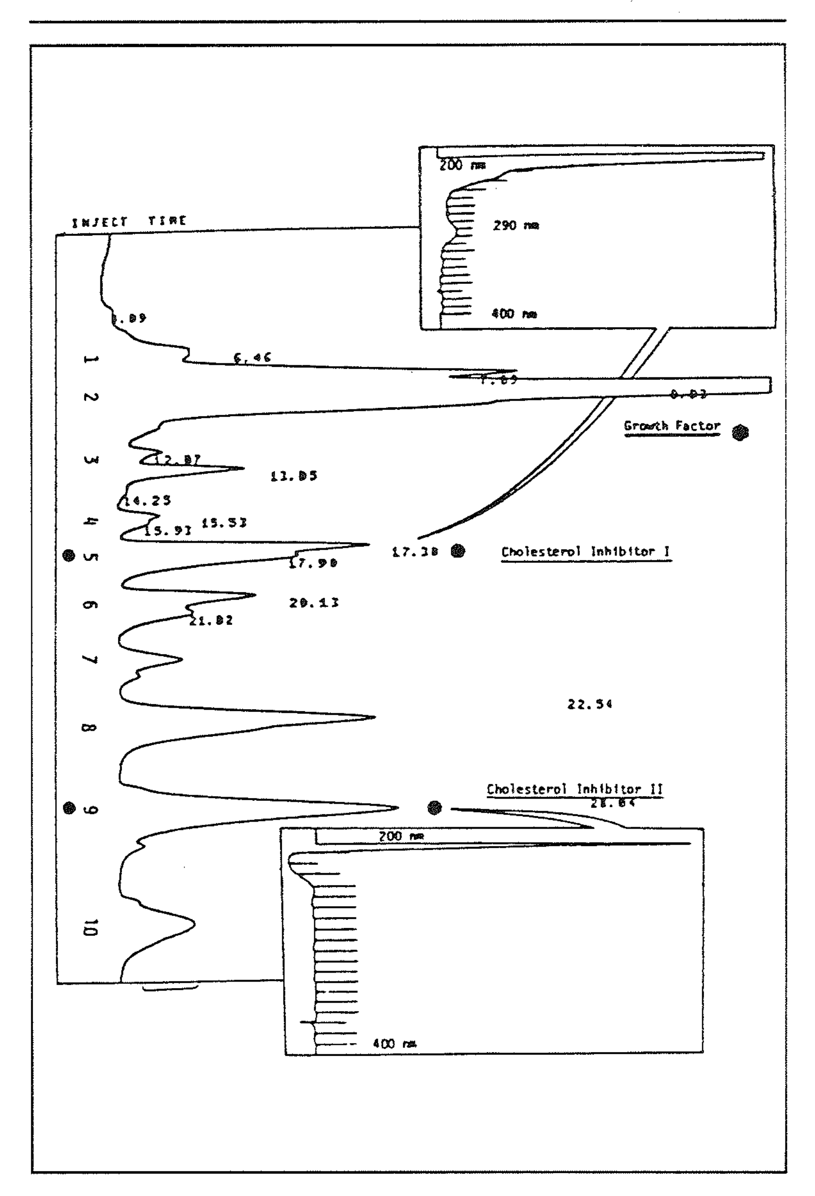


Figure 1: HPLC separation of different components of petoleum ether soluble fraction of HPBF

Figure 2a: Mass spectrum of cholesterol inhibitor I from HPBf

Figure 2b: Ultraviolet absorption spectrum of purified cholesterol inhibitor I.

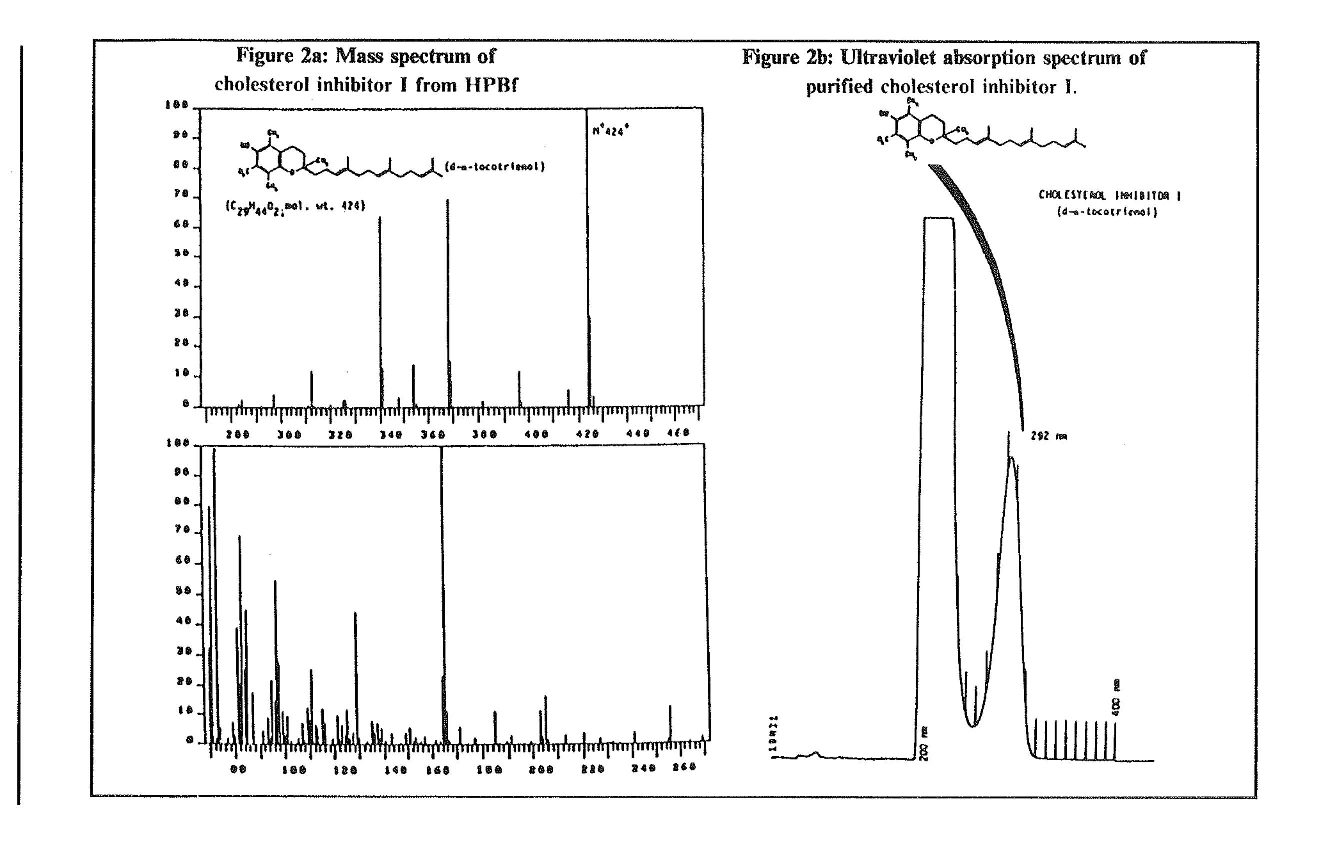
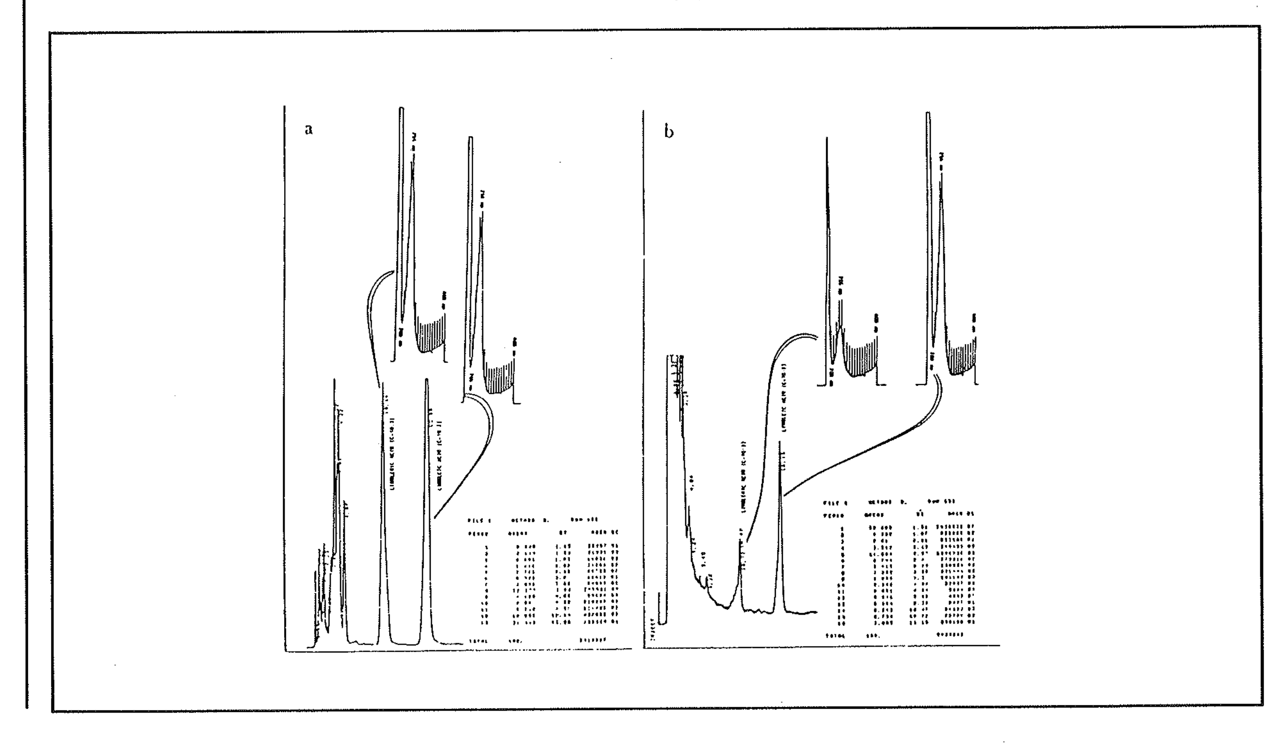




Figure 3: Mass spectrum of cholesterol inhibitor II (m^+ 876^+; $C_{57}H_{96}O_6$) of PESF of HPBFK

Figures 4 (a, b): HPLC of p-bromophenacyl esters derivatives of standard linolenic and linoleic acids (4a) and acids obtained from cholesterol inhibitor II (4b) $M^+ 876^+$) of PESF of HPBF



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EFFECT OF SOME FOLK MEDICINES ON THE URETER
(An experimental study)

Prof. (Drs.) Fahim Abdel Rahim, Ahmed F. Elzayat, Ismail M. Khalaf, Mohamed El-Feky, M. Mansour, Ismail Mahmoud and M. Mabrouk

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EGYPT

INTRODUCTION

Since ancient times man has used herbs and local flora to alleviate many of his diseases and body disorders. The value of plants as drugs is due to the presence of active substances in such plants inducing organic influences in the human body. These substances can be alkaloids, tannines, etc (Mahmoud[1]). Although an immense number of synthetic drugs are used in the treatment of patients with renal colic and urinary calculi, yet some of these drugs have unwanted ill effects. This stimulated us to study the effectiveness of the so called folk medicine agents occasionally used in the treatment of renal colic. In this work a study of the pharmacologic effects of some famous folk medicine drugs in common use were tested on the intact dog ureter. This could be a primary step before the study in humans.

MATERIAL AND METHODS

Experimental Model:

A total of 35 adult mongrel male dogs weighing from 10 to 12 kg were used for acute experiments. A forearm vein was used to give thiopentone sodium (Pentothal) anaesthesia. This was given at a dose of 25 mg/kg. Stability of the anaesthesia was maintained by injection of 25 mg of 5% pentothal solution every 1/2 hour or as

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needed. A femoral vein was exposed and cannulated for continuous saline infusion at a rate of 5 drops/minute. The abdomen was opened through a mid line incision. The intestines were packed to one side and covered with warm saline towels. The left kidney was mobilised and freed from its surrounding fat so as to abolish the effect of respiratory movements on the ureteral tracings. The bladder was opened anteriorly to identify both ureteric orifices. A 6 french ureteral catheter was passed via the right ureteric orifice upto the renal pelvis. A silk ligature was applied around the lower part of the right ureter; which was exposed extravesically, to prevent leakage of urine around the catheter. This catheter was used to collect the urine into a graduated test tube. Another ureteric catheter (4F) was passed upto the left ureter for a distance of 10 cm and was fixed in this position by a stitch applied around the ureteric orifice in the bladder wall. The distal end of this catheter was connected to a pressure transducer coupled to a multichannel recorder (R. 511 A, Beckman).

Ryle tube was passed to the stomach of each dog via the nose. Its position was verified by palpation of the tip of the tube in the stomach through the open abdomen. Saline-wash of the stomach was done to evacuate the gastric contents before administration of the drugs.

Experimental Protocol:

A period of half an hour was allowed for stabilization of the animal to obtain a stable recording of ureteric peristalsis. The frequency of ureteral peristalsis per minute, basal pressure and amplitude of contraction and urine volume were recorded for 1/2 hour and were used as control before administration of the drugs.

The following drugs were given:

1) Zea maize hair extract (glycoalkaloid).

The extract was prepared from Zea maize hair in the Faculty of Science, Al-Azhar University. It was given intravenously in a bolus dose of 0.5 mg/kg in 5 experiments.

2) *Jerusalem stone (Zitonet Israel):* (5 experiments)

This stone was ground to a powder. 5 gms of the powder were dissolved in 25 ml of fresh lemon juice. The solution was given via the Ryle tube at frequent doses of 2 ml every 15 minutes until an effect was noticed on the tracings of ureteral peristalis.

After the end of each experiment the stomach was opened to verify the absorption of the injected drug.

3) *Cymbopogon proximus (Halphabar):* (5 experiments)

This was prepared by adding 5 gms of the dry plant to 500 ml water. The solution was boiled to obtain a decoction of 100 ml volume. Only 2 ml of this solution was injected into the lumen of the stomach via the Ryle tube, the dose was gradually increased and the effect on the ureteral peristalsis and urine volume were recorded.

4) *Ammi visnaga:* (5 experiments)

Five grams of *Ammi visnaga* seeds were added to 500 ml water and boiled to obtain a decoction of 100 ml volume. 2 ml of the decoction were given via the Ryle tube. The dose was gradually increased and the effects on the ureteral activity and urine out-put were studied.

5) *Ambrosia maritima:* (5 experiments)

The decoction was prepared by boiling 5 grams of the dried plant in 500 ml water until a decoction of 100 ml volume was obtained. 2 ml of the decoction were injected in the Ryle tube. The dose was gradually increased and changes in the ureteral peristalsis and urine volume were noted.

6) *Barley:* (5 experiments)

Five grams of the seeds were boiled in 500 ml water to obtain solution of 100 ml volume. 2 ml of the decoction was injected in the

Ryle tube. The dose was gradually increased and the effects on the ureteral activity and urine volume were studied.

7) *Petreselinum crispum*: (5 experiments)

The decoction was prepared by boiling 5 grams of the green plant in 500 ml water to obtain a solution of 100 ml volume. 2 ml of the decoction was given via the Ryle tube. The dose was gradually increased and changes in the ureteral activity and urine out-put were recorded.

In each experiment the changes in the ureteral peristalsis and urine volume were continuously monitored for a period of 3 hours after each drug administration. This period was chosen arbitrarily since the stability of the vital signs of the animal under anaesthesia were affected after 5 hours as a maximum period.

RESULTS

A) Effects on ureteral peristalsis:

Are shown in Tables (1 and 2) and Figures (1, 2, 3, 4, 5, 6, 7).

* *Zea maize hair extract*: It produced a significant decrease in the frequency, basal ureteric pressure and amplitude of ureteral peristalsis.
* *Jerusalem stone (Zitonet Israel)*: Significant increase in the frequency, basal pressure and decrease in the amplitude was noted after drug administration.
* *Cymbopogon proximus (Halphabar)*: It elicited significant decrease in the basal pressure and amplitude of peristalsic waves and insignificant decrease in the frequency of ureteral contractions.
* *Ammi visnaga*: Significant increase in the basal pressure was observed following drug administration without any significant decrease in the frequency and amplitude of ureteral peristalsis.
* *Ambrosia maritima*: The drug produced significant decrease in

the frequency of ureteric peristalsis and the basal pressure but had no significant effect on the amplitude of ureteric peristalsis.

* *Barley*: Significant elevation of the basal pressure and significant decrease in the amplitude of the ureteral peristalsis was observed.
* *Petroselinium crispum*: Produced significant decrease in the frequency and amplitude and significant increase in the basal pressure.

B) Effects on the urine volume:

All tested drugs except *Halphabar* produced a significant increase in urine out-put.



Fig. 1: Effect of Glycoalkaloid on Ureteral Peristalsis in dogs



Fig. 2: Effect of Jerusalem stone (Zitonet Israel) on Ureteral Peristalsis in dogs

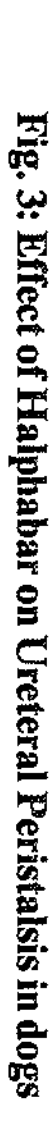


Fig. 3: Effect of Halphabar on Ureteral Peristalsis in dogs



Fig. 4: Effect of Ammi visnaga on Ureteral Peristalsis in dogs

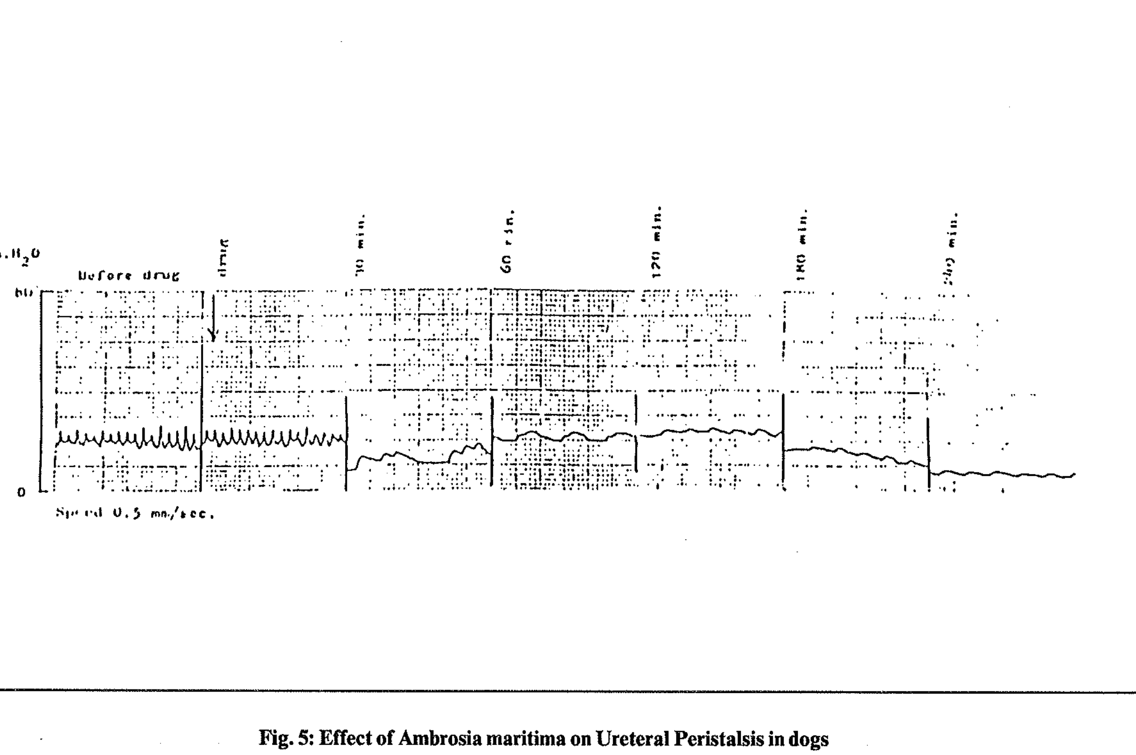


Fig. 5: Effect of Ambrosia maritima on Ureteral Peristalsis in dogs

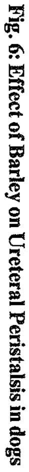


Fig. 6: Effect of Barley on Ureteral Peristalsis in dogs

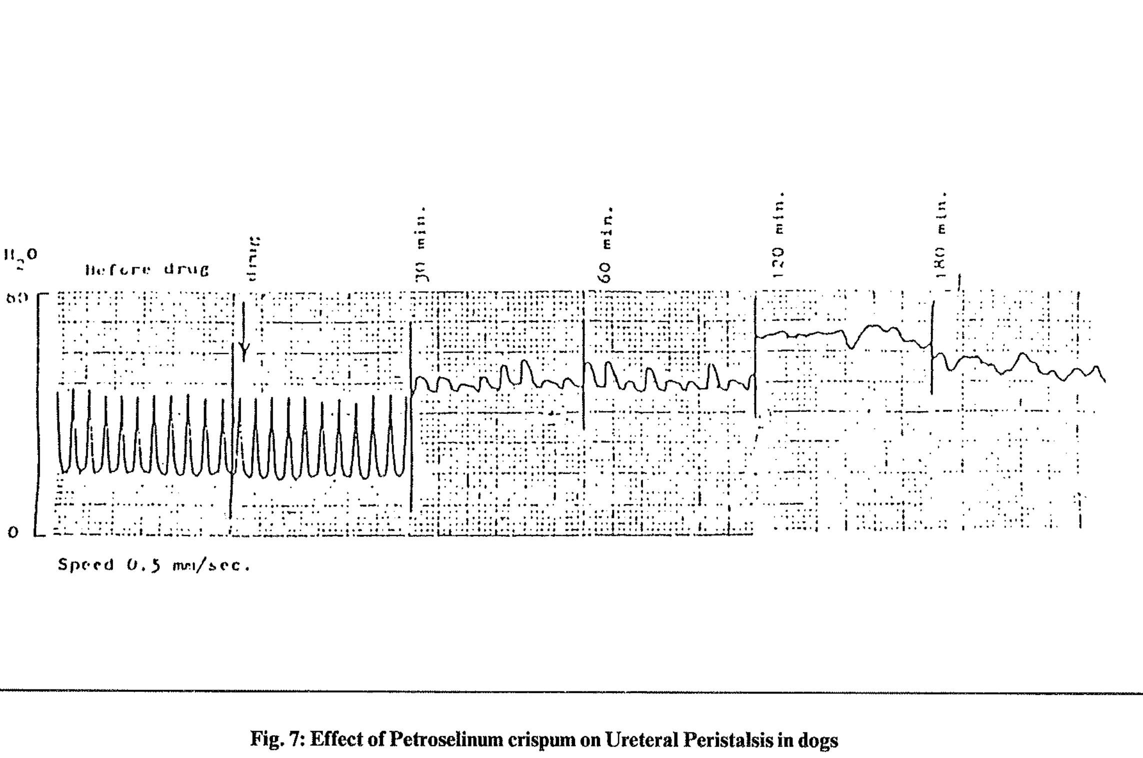


Fig. 7: Effect of Petroselinum crispum on Ureteral Peristalsis in dogs

TABLE (1)

EFFECT OF SOME FOLK MEDICINE DRUGS ON URETERAL PERISTALSIS

(Mean + S.E; n=5).

Drug	Parameter	Before injection	After injection (one hour)
* Zea maize hair extract (Glycoalkaloid)	F	8 ± 0.7	5 ± 0.44#
	B	16.6 ± 0.72	14 ± 0.44#
	A	11.4 ± 0.81	8.8 ± 0.37#
* Jerusalem stone (Zitonet Israel)	F	7.4 ± 0.29	12.6 ± 0.72#
	B	24.8 ± 1.36	32.8 ± 1.5#
	A	10.2 ± 0.41	5.6 ± 0.5#
* *Cymbopogon proximus* decoction (Halpha bar)	F	13.4 ± 1.2	11.6 ± 1.2
	B	29.8 ± 1.39	23.2 ± 0.89#
	A	4.9 ± 0.4	8.2 ± 0.48#
* *Ammi visnaga* decoction	F	15.6 ± 0.67	14.8 ± 1.3
	B	24.8 ± 1.2	30 ± 0.63#
	A	5.6 ± 0.4	5 ± 0.44
* *Ambrosia maritima* decoction	F	10.6 ± 0.68	9 ± 54
	B	13.2 ± 0.74	13.2 ± 0.74
	A	5.2 ± 0.36	4.1 ± 0.36
* Barley decoction	F	10.2 ± 0.8	9.6 ± 0.24
	B	28 ± 0.83	36 ± 7.1#
	A	8.8 ± 0.58	7 ± 0.63#
* *Petroselinium crispum*	F	25.6 ± 0.24	7.6 ± 0.5#
	B	19.8 ± 0.66	45.8 ± 1.75#
	A	8.6 ± 0.67	7 ± 0.38#

#: Statistically significant difference from preinjection level.

F : Frequency of peristalsis/minute.

B: Basal ureteric pressure in cm H_2O

A: Amplitude of peristaltic contraction in cm H_2O

TABLE (2)

CHANGES IN URINE VOLUME AFTER ADMINISTRATION OF DRUGS (n=5)

Drug:	Urine volume in ml/hour mean ± S.E.		
	Before injection	After one hour	After 3 hours
*Zea maize hair extract (glycoalkaloid)	12 ± 0.6	19.9 ± 0.6	14.6 ±9#
* Zitonet Israel	12 ± 9	14.6 ± 0.9	20.6 ±9#
* *Cymbopogon proximus* (Halphabar)	12.5 ± 0.48	12.7 ± 0.5	11.5
* *Ammi visnaga*	11.6 ± 0.8	12 ± 0.7	13.8 ± 0.6#
* *Ambrosia maritima* decoction	13.4 ± 0.4	19.1 ± 0.4	24.3 ± 0.9#
* Barley decoction	14.2 ± 0.4	18.5 ± 0.4	20 ± 0.4#
* *Petroselinum cryspum* decoction	14.3 ± 0.7	15.3 ± 0.3	18.7 ± 0.8#

#: Statistically significant difference from preinjection level.

DISCUSSION

The occasional unwanted ill-effects of the synthetic drugs used in the treatment of renal colic and urinary calculi coupled with the known success of some folk medicine agents in the relief of these conditions have stimulated us to examine the effect of some folk medicine agents on the ureteral peristalsis in the experimental animals.

The experimental set up used in this work, entails studying the effect of folk medicine drugs on the intact dog ureter. The kidney was mobilised and dissected from the diaphragm to abolish the effect of respiratory movements on tracings of the ureteral peristalsis.

This experimental study is a urodynamic assessment of the changes in the ureteral activity following administration of folk medicine drugs. Simultaneous measurement of the urine volume with the changes in the ureteral activity was done. There are some pitfalls in the experimental set up which included the presence of indwelling ureteral catheter in the ureter, which acts as a foreign body exciting the ureteral peristalsis. However, we observed a decrease in the ureteral activity in some of the tested materials. We agree that simultaneous recording of the blood pressure should be done in any pharmacological experiment, unfortunately this was not done. Nevertheless our results are reproducible and in accordance with the results of other investigators.

Zea maize hair extract:

In this study the extract produced decrease in the frequency, basal ureteric pressure and amplitude of contraction. This decrease in the ureteral activity was observed after drug administration and even after the diuretic action was noted. This effect could explain the antispasmodic effect of the drug. These results are in accordance with the findings of El-Zayat et al.[2,3], who noted a direct inhibitory effect of Zea maize hair extract on the isolated dog ureter and rabbits jejunum. Guerrero[4] proved that Zea maize hair extract has a diuretic action.

Jerusalem stone (Zitonet Israel):

Probably this is the first experimental monitoring of the effect of this important drug among folk medicines on the ureter. In the present work the drug produced significant increase in the frequency of ureteral peristalsis and basal pressure, with a reduction in the amplitude of contraction. In addition there was evident increase in the urine out-put in the first hour and up to three hours. The increase in the frequency and basal pressure could be attributed to the diuretic action of the drug, while the decrease in

the amplitude could be due to the antispasmodic action of the nitrogenous bases. Abdel Rahim et al.[5] found that the drug was beneficial in the management of renal colic and urinary calculi as it assisted the passage of the ureteric and renal stones. They suggested that the diuretic action to the drug could be possibly due to its lithium carbonate content, while the antispasmodic action to the nitrogenous bases.

Cymbopogon proximus (Halphabar):

Significant decrease in the basal ureteric pressure and amplitude of contraction was noted on testing the drug. This decrease in the ureteral activity could be explained by the antispasmodic action. Ghaleb[6] found that halphabar has antispasmodic action, producting inhibitory effect on the isolated intestine and guinea pig ileum.

Ammi visnaga:

This drug has long been used in Egypt as an antispasmodic because of its direct action on the smooth muscle fibres (Helmy)[7]. In 1967, Mahran[8] proposed that an aqueous infusion of *Ammi visnaga* has an antispasmodic and diuretic effects. On the other hand Al-Bialy[9] noted that Khellin was completely disappointing in most of his cases. In the present work although significant increase in the urine volume was observed with *Ammi visnaga*, yet there was no increase in the frequency or amplitude of ureteral persistalsis. This could be attributed to the antispasmodic action of the drug. On the other hand elevation of the basal pressure could possibly be due to its diuretic effect.

Ambrosia maritima L (Damssissa):

On the basis of the famous clinical effect amongst folk medicines as antispasmodic, the drug was tested experimentally. There was decrease in the ureteral activity although the decoction produced evident diuretic effect after one and up to three hours.

This decrease in the ureteral activity could possibly be explained by the antispasmodic action of the nitrogenous bases or alkaloids and volatile oils in the plant. These findings are in accordance with Sorm[10] and Mahran[8] who found that it has antispasmodic and diuretic action.

Barley:

Mahran[8] postulated that the aqueous infusion and decoction of barley has a diuretic effect. In this work an increase in the basal pressure and decrease in the amplitude of ureteral contraction were noted. The increase in the basal pressure could be due to the prominent diuretic effect of the decoction. Reduction in the amplitude of ureteral peristalsis could possibly be due to the antispasmodic action.

Petroselinum crispum:

Aliev et al.[11] mentioned that the plant contains alkaloids, glycosides, volatile oils, organic acids, vitamin C and K. It has a hypotensive action. However, it increased the amplitude of the heart beat. This effect was attributed to depressant action on the central nervous system. Watt et al.[12] found that the active component in the fruit is a piol which has abortifacient effect through stimulation of uterine tissue. Moreover, Franswarth et al.[13] attributed the carminative and antispasmodic action to the volatile oils in the plant.

In this work significant decrease in the frequency and amplitude with increase in the basal ureteric pressure and increase in urine output were noted. The decrease in the ureteral activity could be due to the antispasmodic action of the volatile oils and alkaloids in the plant. The elevation in the basal pressure could be attributed to the diuretic effect of the drug.

These observations on the effect of folk medicine agents are preliminary. Further study is recommended to investigate the effect

of each agent used, and its active ingredient(s), on ureteral function. The present results indicate that some folk medicine agents have an inhibitory effect, while others have stimulatory action on ureteral activity. Moreover most of the tested agents produced a diuretic effect. These actions need to be studied in human volunteers before explorating their effect in clinical situations.

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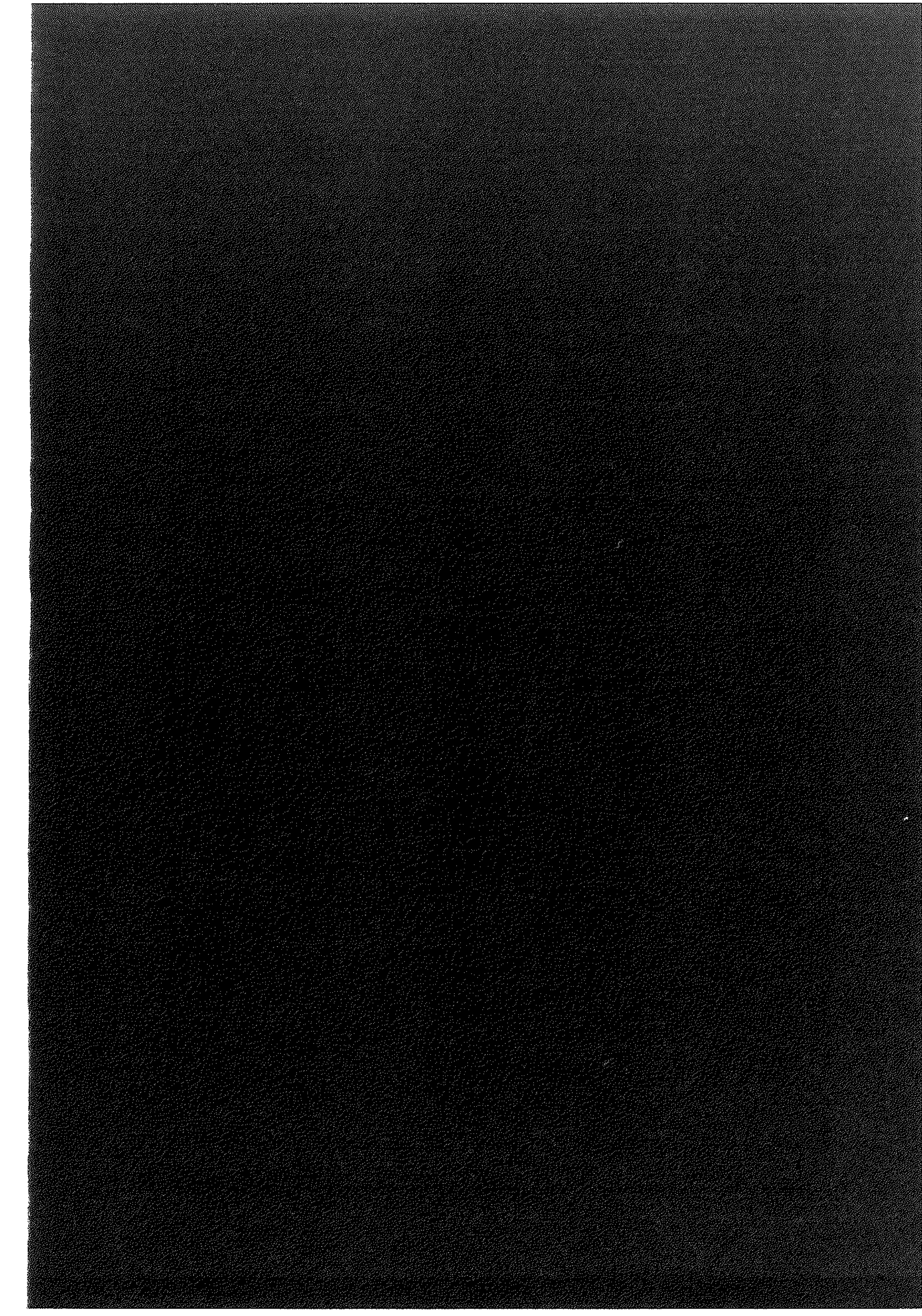
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PRELIMINARY CLINICAL EVALUATION OF CERTAIN HERBAL REMEDIES IN THE TREATMENT OF VITILIGO

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INTRODUCTION

Vitiligo *(Bars)* has been a well known disease to our Islamic Medical figures. Al-Tabari (810-895 AD)[1] has described the aetiology of the disease. Al-Rhazi (850-925 AD)[2] has given a comprehensive description of *Bars*, Ib-Sina (980-1037 AD)[3] has mentioned, in his famous book, Al-Qanoon, the role of tissue metabolism in *Bars* and mentioned that the disease can be transmitted from parent to off-spring.

Today, vitiligo is considered a common skin disorder characterized by gradual development of depigmented white patches of varying size and shapes anywhere on the skin and mucous membrane. It affects about 1% of the population and both sexes are equally affected[4]. Its onset could be at any age, but usually it is in the first decade[5]. The distribution of the lesions are multiple, bilateral and symmetrical in most of the cases. When they occur on the exposed part of the body i.e. face and back of the hands, they often lead to considerable cosmatic, social and psychological difficulties. As a result, the patients are generally disturbed mentally and may develop inferiority complex.

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The exact cause of vitiligo is still unknown despite centuries of speculation. Three major theories have been proposed to explain the pathogenesis of the obscure disease[6] - the autodestruction theory, the auto-immune theory and the neural theory.

The histopathology of vitiligo is also not quite clear and the only change is the disappearance of melanocytes in the depigmented area[7].

There is no specific treatment for vitiligo. The most widely used are photo-chemotherapy (PUVA)[8] and corticosteroids[9]. Both of these treatments are unsatisfactory and not free from serious side effects[10].

From the review of literature, it becomes evident that vitiligo in all its aspects; pathogenesis, pathology, clinical picture and therapy presents a considerable problem in medicine. All these facts stimulated our interest to study this disease with a hope to find a new approach in the treatment of this recalcitrant disease. The aim of study was to evaluate the therapeutic efficacy and safety of certain herbal remedies in the treatment of vitiligo *(Bars)*.

MATERIAL AND METHODS

184 patients with vitiligo were selected from the outpatient clinic of Islamic Centre for Medical Sciences, Kuwait. Their age ranged between 12-60 years, 94 of them were females and 90 males. They were subjected to thorough clinical examinations and laboratory investigations, including stool and urine analysis, complete blood count, sedimentation rate, fasting blood sugar, renal function test, liver function test, lipid profile and serum electrolytes. These investigations were done for every patient on their entry to the clinical trial and after every three months. The criterion for selection was to include such cases of vitiligo which

were free from other systemic diseases. The base line data were recorded on a case sheet prepared for the purpose of the study and the depigmented areas of the body were marked out on a human sketch.

Photographs and coloured slides were taken for most of the cases before and after the treatment.

The patients were divided into three treatment groups. The first group was given oral medicine (PS) and topical application paste (Z1). The second group was given the same oral medicine (PS) and paste (Z2) for topical application and the third group was given the same oral medicine (PS) and paste (Z3) for topical application (Table 1). The patients were exposed to sun light for 5-10 minutes depending upon their tolerance.

TABLE 1: DISTRIBUTION OF THE PATIENTS ACCORDING TO THE TREATMENT GROUP

Groups	Number of patients	Male	Female	Treatment
1st	24	12	12	PS + Z1
2nd	65	30	35	PS + Z2
3rd	95	48	47	PS + Z3
TOTAL	184	90	94	

- The dosage of PS capsules orally given to all the patients, was two capsules in the morning and two capsules in the evening after meals, with the total dose of 3 gm.
- The application of the pastes: Z1, Z2 or Z3, was done by mixing a small amount of powdered drug with water and applied on all the existing white patches. The time of retaining the paste on the patches varied from 30-60 minutes. Afterwards the paste was washed off.

The patients were put also on a special diet according to the traditional system of medicine[11], some food articles were restricted and some recommended.

Clinical follow-up was done monthly and the response to the treatment was evaluated every 3 months.

Criteria for assessment of response

Response to the treatment was assessed on the following lines:

1 - No response: no pigmentation, and appearance of new lesions.
2 - Response:

1. good response (41 - 100% repigmentation).
2. fair response (up to 40% repigmentation).
3. poor response (no repigmentation but no new lesion developed and no increase in the size of the existing patches observed).

RESULTS

The preliminary results of the work are presented under the following headings:

I) General Observations

II) Therapeutic Response

I) General Observations

Of the 184 cases, who were selected in this study, the females were 94, while the males were 90; so the ratio of female to male was 1:1.04. The age of the patients ranged between 12 to 60 years. The duration of the disease varied from 1 month to 28 years. The number of localized type of the disease was 150 cases while the generalized type was 34 cases (Table 2).

TABLE 2: DISTRIBUTION OF THE PATIENTS ACCORDING TO THE TREATMENT GROUP

Group	1st Group	2nd Group	3rd Group	Total
Patients	24	65	95	184
Male	12	30	48	90
Female	12	35	47	94
Age	12 - 47Y	12 - 56Y	12 - 60Y	12 - 60Y
Duration	2M - 12Y	1M - 20Y	1M - 28Y	1 - 28Y
Types:				
- Localized	19	50	81	150
- Generalized	5	15	14	34

II) Therapeutic Response

Group No. 1 = PS Capsule + Z1 paste.

In this group, the drugs were tried on 24 cases. The distribution of patients according to sex, age group, duration of the disease and the clinical picture is shown in Table 2.

The therapeutic response is detailed as follows:

i) General therapeutic response.
ii) Response in relation to sex group.
iii) Response in relation to clinical type.
iv) The side effects of the treatment.

i) *GENERAL THERAPEUTIC RESPONSE:*

Out of 24 cases of the group, 22 cases (92%) responded to the treatment while 2 cases (8%) did not respond.

In the responding cases, 7 cases (29%) showed poor response, 7 cases (29%) showed fair response, while 8 cases (34%) showed good response. Table 3 shows the general response in Group I treatment.

TABLE 3: GENERAL THERAPEUTIC RESPONSE TO TREATMENT

Response	Nil	Poor	Fair	Good	Total
No. of patients	2	7	7	8	24
Percentage	8%	29%	29%	34%	100%

ii) *RESPONSE IN RELATION TO THE SEX GROUP:*

The fair and good response was more in females'group than in males' group. Table 4 shows the response in relation to sex group.

TABLE 4: RESPONSE IN RELATION TO SEX GROUP

Response	Nil	Poor	Fair	Good	Total
Male	2	5	3	2	12
Female	0	2	4	6	12
Total	**2**	**7**	**7**	**8**	**24**

iii) *RESPONSE IN RELATION TO THE CLINICAL TYPES:*

It was observed that all the cases with good response had localized vitiligo. The fair response was observed in 6 localized type and in one generalized type. Poor response was found in 4 cases with generalized types and 3 cases with localized types. No response was found in 2 localized type (Table 5).

TABLE 5: RESPONSE IN RELATION TO THE CLINICAL TYPE

Response	Nil	Poor	Fair	Good	Total
Localised	2	3	6	8	19
Generalized	0	4	1	0	5
Total	**2**	**7**	**7**	**8**	**24**

iv) *SIDE EFFECT OF THE TREATMENT:*

In Group 1-trial, no side effect was observed during the treatment.

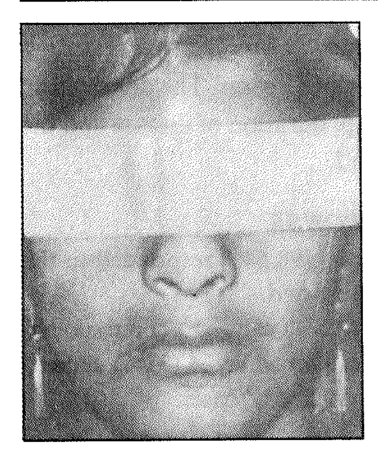


Fig. 1: Before treatment.

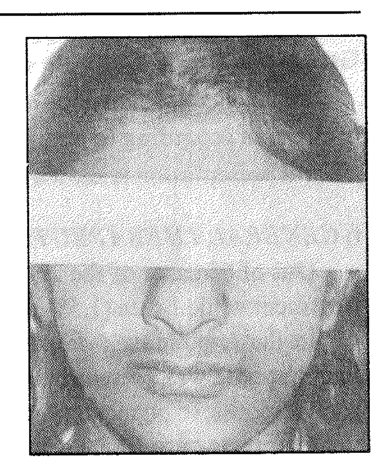


Fig. 2: After treatment.

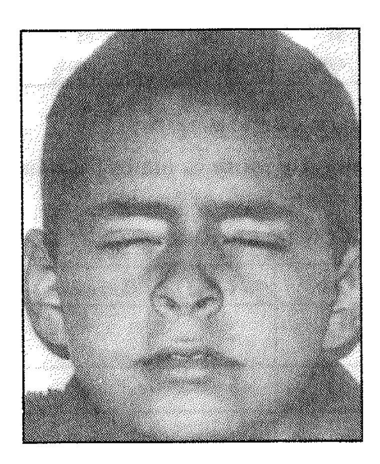


Fig. 3: Before treatment.

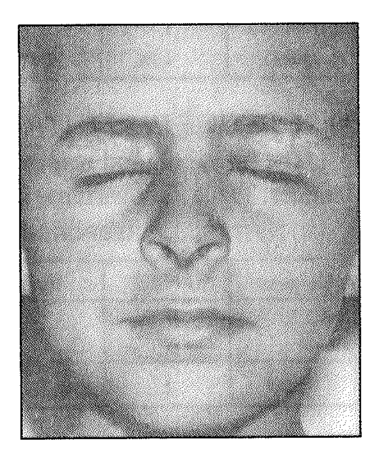


Fig. 4: After treatment.

Fig. 1, 2, 3 and 4 show the response in the 1st group. PS + Z1

Group No. 2 = PS Capsule + Z2 paste.

The drugs were tried in this group on 65 cases. The distribution of patients according to sex, age group, duration of disease and the clinical presentation is shown in Table 2.

i) *GENERAL THERAPEUTIC RESPONSE:*

Out of 65 cases of the 2nd group, 64 (98.5%) responded to the treatment while 1 case (1.5%) did not respond.

In the responding cases, 8 cases (12.5%) showed poor response. Fair response was present in 32 cases (49%) while 24 (37%) showed good response; so pigmentation appeared in (86%) cases.

Complete cure 100% occurred in 4 patients. Table 6 shows the general therapeutic response.

TABLE 6: GENERAL THERAPEUTIC RESPONSE

Response	Nil	Poor	Fair	Good	Total
No. of patients	1	8	32	24	65
Percentage	1.5%	12.5%	49%	37%	100%

ii) *RESPONSE IN RELATION TO THE SEX GROUP:*

The fair and good response was slightly more in males than in females. Table 7 shows the response in relation to sex group.

TABLE 7: RESPONSE IN RELATION TO SEX GROUP

Response	Nil	Poor	Fair	Good	Total
Male	0	1	17	12	30
Female	1	7	15	12	35
Total	**1**	**8**	**32**	**24**	**65**

iii) *RESPONSE IN RELATION TO CLINICAL TYPES:*

It was observed that out of 24 cases of good response, 23 cases had localized type of Vitiligo while 1 had generalized type. The fair response was observed in 22 localized types and in 10 generalized types. Poor response was found in 3 localized type and in 5 generalized type. No response was found in one localized type (Table 8).

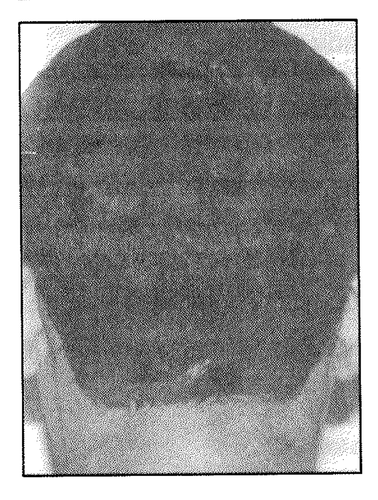


Fig. 5: Before the treatment.

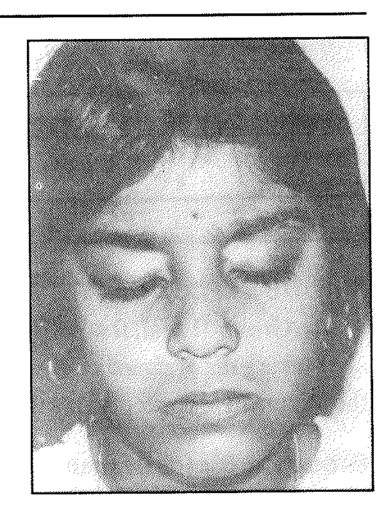


Fig. 7: Before the treatment.

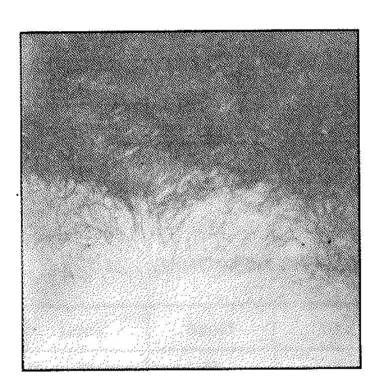


Fig. 6: After the treatment.

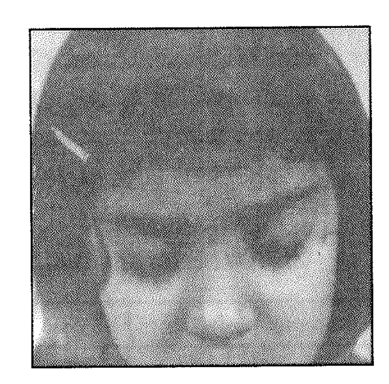


Fig. 8: After the treatment.

TABLE 8 : RESPONSE IN RELATION TO CLINICAL TYPE

Response	Nil	Poor	Fair	Good	Total
Localized	1	3	22	23	49
Generalized	0	5	10	1	16
Total	**1**	**8**	**32**	**24**	**65**

iv) *SIDE EFFECT OF THE TREATMENT:*

In Group-II trial, few patients complained of epigastric discomfort after taking the oral medicine, which was improved after several days of the treatment and after taking the medicine after meals.

Group No. 3 = PS Capsule + Z3 paste.

In this group the drugs were tried on 95 cases. The distribution of patients according to sex, age group, duration of disease and the clinical presentation is shown in Table 2.

i) *GENERAL THERAPEUTIC RESPONSE:*

Out of 95 cases, 93 (98%) cases responded to the treatment while 2 (2%) did not respond.

In the responding cases, 17 cases (18%) showed poor response, 31 cases showed fair response (33%), while 45 (47%) cases showed good response. So pigmentation occurred in 76 cases (80%).

Complete cure (100%) was observed in 3 cases (Table 9).

TABLE 9: GENERAL THERAPEUTIC RESPONSE

Response	Nil	Poor	Fair	Good	Total
No. patients	2	17	31	45	95
Percentage	2%	18%	33%	47%	100%

ii) *RESPONSE IN RELATION TO SEX GROUP:*

The fair and good response was more in males than in females, while poor response was more in females than in males (Table 10).

TABLE 10: RESPONSE IN RELATION TO SEX GROUP

Response	Nil	Poor	Fair	Good	Total
Male	1	7	19	24	51
Female	1	10	12	21	44
Total	**2**	**17**	**31**	**45**	**95**

iii) *RESPONSE IN RELATION TO THE CLINICAL TYPE:*

It was observed (Table 11) that, 38 cases of good response had localized vitiligo and 7 cases had generalized type.

In fair responded cases, 29 had localized type while 2 cases had generalized type. In poor responded cases, 12 had localized type while 5 had generalized type.

One localized type and one generalized type did not respond to treatment.

TABLE 11: RESPONSE IN RELATION TO CLINICAL TYPES

Response	Nil	Poor	Fair	Good	Total
Localized	1	12	29	38	80
Generalized	1	5	2	7	15
Total	**2**	**17**	**31**	**45**	**95**

iv) *SIDE EFFECTS OF THE TREATMENT:*

No significant side effects were observed in this group. Some cases developed contact irritation from the topical application which was usually improved after discontinuing the medicine for few days, and increasing the dilutions of the paste. Some patients complained of epigastric discomfort after taking the oral medicine. These symptoms decreased with time and by taking the medicine after meals.

The general therapeutic response in the total of 184 patients

Out of 184 cases, 5 cases (3%) did not respond to the treatment while 179 cases (97%) responded to the treatment.

In the responding cases, 32 cases (17%) showed poor response; 70 cases (38%) showed fair response, while 77 cases (42%) showed good response. The results are shown in Table 12.

Complete cure was achieved in 7 cases (4%) and pigmentation occurred in 147 cases (80%).

TABLE 12: THE GENERAL THERAPEUTIC RESPONSE IN ALL THE CASES STUDIED

Response	Nil	Poor	Fair	Good	Total
Group I	2	7	7	8	24
Group II	1	8	32	24	65
Group III	2	17	31	45	95
Total	**5**	**32**	**70**	**77**	**184**
Percentage	**3%**	**17%**	**38%**	**42%**	**100%**

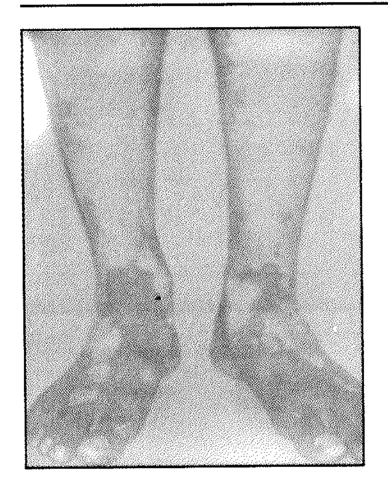


Fig. 9: Before treatment.



Fig. 10: After treatment.

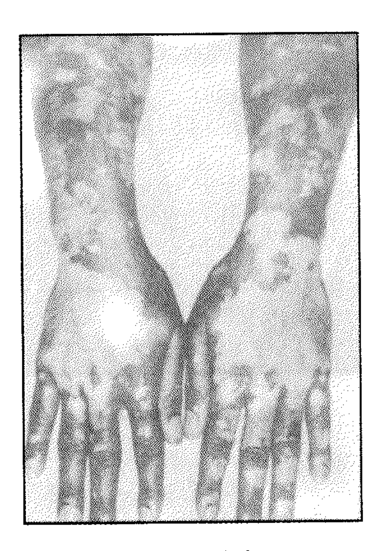


Fig. 11: The same patient before treatment.

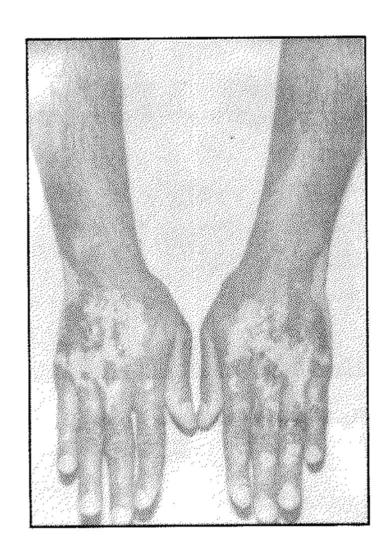


Fig. 12: After treatment.

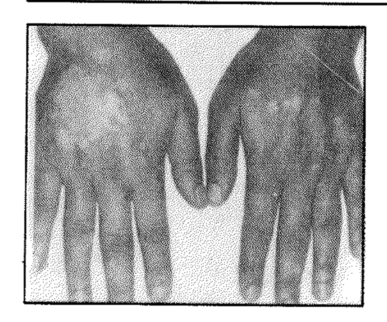


Fig .13: Before treatment.

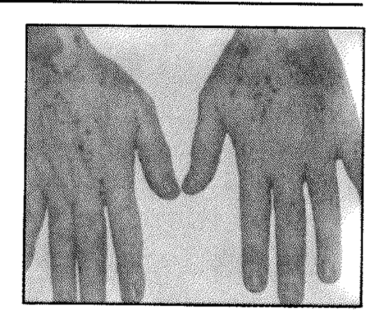


Fig.15: Before treatment.

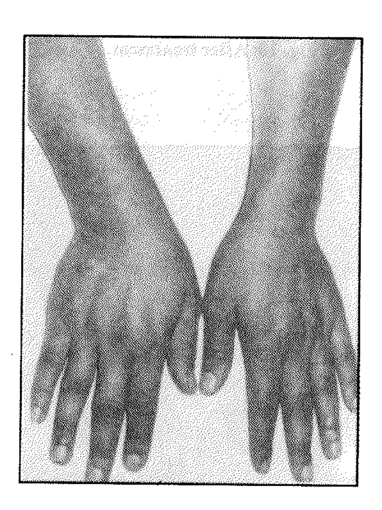


Fig. 14: After treatment.



Fig.16: After treatment.

COMMENT AND CONCLUSION

Vitiligo *(Bars)* is well known to the medical world from time immemorial and has ever been a challenge to the medical profession. It was one of miracles associated with Jesus Christ to cure the patient of *Bars* by the touch of his hand. From that time till now the cure of patient with vitiligo has always been the task of all investigators who worked on this disease. Many attempts had been made for the treatment of vitiligo by Herbal remedies used by Islamic medical workers[12-16], and their results were encouraging.

In our study, the preliminary results of using certain remedies in the treatment of vitiligo showed that all drugs used were effective in the treatment of both localized and generalized vitiligo, irrespective of age, sex of the patients or the clinical presentation. In group I, pigmentation started to appear in 15 cases out of 24 cases, so (63%) of cases gave pigmentation response, while 29% showed poor response. In group II, out of 65 cases 56 cases showed pigmentation (86%) and 3 cases showed complete cure (5%). In group III, pigmentation occurred in 79 out of 95 cases (83%) and complete cure was seen in 4 cases.

From the above results we observed that out of 184 cases, complete cure (100%) was achieved in 7 cases (4%), 77 cases (42%) showed good response and 70 cases showed fair response (38%). While no pigmentation occurred in 37 cases (20%), 147 cases (80%) showed pigmentation.

Our preliminary results showed that in all treatment groups, the general response was good and encouraged us to continue and extend our trial.

In the follow up of patients no changes were observed in the investigations which were done every 3 months, no significant side effects were found during the treatment apart from few cases developing local irritation for the topical applications especially Z3 paste which was improved after the dilution of the paste.

From the above mentioned results, our study showed that the herbal remedies used in this study were effective in the treatment of vitiligo *(Bars)* and were safe and no side effect was noticed.

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TREATMENT OF BARS (VITILIGO) WITH ARAB MEDICINE

Mohd. Iqbal Ali, M.M. Ali Khan, Bahauddin and Mastan Ali

INDIA

TREATMENT OF BARS (VITILIGO) WITH ARAB MEDICINE*

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INDIA

Abstract

Two Arab drugs, 'Safoof and Zamad' were tried in 1,745 cases, orally and topically, in separate groups. In 445 cases, only Zamad was used and in 1,300 cases Safoof and Zamad were tried. The results were found to be satisfactory in both the groups. Zamad alone yielded 75.74% response whereas Safoof and Zamad, combined yielded 84.15% response. This study has shown that Safoof and Zamad are very effective in the management of cases of vitiligo.

INTRODUCTION

Bars is an Arabic term used for Vitiligo, an idiopathic variety of leucoderma. This achromia of the skin may be limited to few patches or it may be extensive and widespread. When extensive, it is often bilateral and adopts symmetry in distribution. It can appear on any part of the body but the common sites are the face, dorsal portions of fingers, hands, waist, legs, toes and feet. Mucous membranes may also be affected. (Rabbin Tabri[1], al-Razi[2], Ibn Sina[3], Azam Khan[4], Lerner[5,6], Behl[7], Savil[8], and Macky[9]).

Bars was known even in the period of "Aushooryan" in 2200 B.C. (Mahmood[10]). The description of *Bars* is found in Atherveda (1400 B.C.). Al-Razi[2], gave a comprehensive description of *Bars*. He attributed it to excess of Khilth-e-Balgam, a humoral disturbance, and

* Bulletin of Islamic Medicine, 1: (454-461), 1981.

Zufe-Hazm (weakness of digestion) and coldness of blood. Ibn Sina, Tabri, Masihi, Gilani and other eminent Hakims agree with this view (Ibn Sina[3], al-Razi[2], Rabban Tabri[1] and Azam Khan[4]). Buqrat and Jalinoos were also of the same opinion about its causation. Ibn Sina in his "al-Qanoon" said that *Bars* is hereditary, and it is due to failure of the power which gives shape to tissues (Ibn Sina)[3].

Exact cause of *Bars* is unknown; however, various causes from heredity to viral infection have been attributed to this disease. (Anderson[11], Behl[7]). Metabolic errors and dietary deficiencies are held responsible (Ibn Sina[3], al-Razi[2], Behl[7]). Gastrointestinal disorderers - e.g. intestinal worms, chronic amoebiasis, giardiasis and chronic dyspepsia may be counted as precipitating factors. Mental stress and frequent use of broad-spectrum antibiotics have also been noted as predisposing factors in some cases (Behl[7]). Endocrine disorders and auto-immunity are also accused. Trauma and constant pressure may cause depigmentation in susceptible persons. Use of incompatible food articles such as simultaneous use of milk and fish, is also described as its cause by ancient Hakims. Excess use of sour things, oranges, lemons and tamarind, and food touched by rats, cats and certain other animals are also held responsible as its cause (Ibn Sina[3], al-Razi[2], Rabban Tabri[1], Azam Khan[4]). Drinking or bathing in water - warmed by direct exposure to sun rays has been described as one of the causes (al-Hadith).

Intense research has been carried out on vitiligo in different parts of world. Much has been explored, still its etiology remained obscure and the treatment discouraging. "The repigmentation is rare in vitiligo, total response is seen in 10 to 15 per cent of the cases". Mercus A - Krupp[12].

It has been a social scourge since time immemorial and now its incidence is increasing. The subjects suffering from vitiligo develop inferiority complex and avoid societies. They are psychologically disturbed.

The study was taken up considering the un-yielding nature of the disease and social importance of its treatment with an object to find out its effective treatment. In this paper the results on therapy of *Bars* using two Arab formulae studied at Central Research Institute for Unani Medicine, Hyderabad, A.P., India are presented.

MATERIALS & METHOD

MATERIALS

1) 1,745 patients of *Bars* attended the Institute.
2) Drugs Used: a SF (Safoof) - for oral use - powder of Babchi seeds (*Psoralea corylifolia* Linn) treated in vinegar الخل for seven days, dried, powdered and preserved for use. b) Z_1 (Zamad) - for external application on *Bars* lesions.

   Zamad - (Ingredients):

   i) Babchi seeds (*Psoralea corylifolia* Linn).

   ii) Geru (Red-ochre).

   iii) Gandak Amlasar (Sulpur)

   iv) Gulnar (*Punica granatum* Linn)

   All the 4 ingredients were powdered separately and mixed in equal quantities to be used as a zamad. The powder of zamad in a required quantity (according to extension of lesions) was mixed with water and a fine paste prepared.

METHOD

On 445 patients only zamad was tried as an external application and on 1300 patients safoof and zamad both, safoof orally and zamad externally. The criteria for selection of patients was to exclude them from all other systemic diseases specially infectious skin diseases which produce achromic patches on the skin and to select pure *Bars* cases.

Dosage: Safoof: 6 gm daily. 3 gm of safoof was given in morning and evening, 15 minutes before meals.
Zamad: The zamad powder in a required quantity mixed and grounded well in water (1 × 5W × V) to be applied on *Bars* lesions. After application of zamad the lesions were exposed to sun from 2 to 10 minutes according to individual tolerance. Exposure to sun was avoided if patients showed intolerance.

The duration of treatment was 3 to 18 months.

The response was judged clinically and also by taking photographs before and after treatment.

Trials with zamad:

Zamad was tried as an external application (without any other oral or topical medicine), in 445 *Bars* patients irrespective of patients age, sex and chronicity of the disease or extension of the lesions.

Observation and results.

The duration of illness varied in these 445 cases from 1 month to 30 years and above as shown in Table I.

It was noted that majority of these cases had a long duration of illness before they attended the Institute for treatment.

The duration of treatment with zamad ranged from 3 to 12 months and above, shown in Table II.

Response to the treatment with zamad:

Out of 445 Bars cases, 337 responded to the given treatment with zamad and the rest (108) did not show any response, i.e. 75.74% of the cases showed good response to the treatment. The results are shown in the Table III.

TABLE I

Showing the duration of illness in 445 Bars cases treated with ZAMAD

DURATION OF ILLNESS	NO. OF PATIENTS
1-11 months	156
1 -5 years	189
6 -10 years	57
11 - 15 years	21
16 - 20 years	13
21 - 25 years	4
26 - 30 years	5
TOTAL	445

TABLE II

Showing the duration of treatment

DURATION OF TREATMENT	NO. OF PATIENTS
1-3 months	200
4-6 months	133
7 -9 months	39
10 -12 months	29
Above 1 year	44
TOTAL	445

TABLE III

Showing the response to the treatment with Zamad

100% Cure	91 -99% Cure	71 - 90% Cure	51 - 70% Cure	41 -50% Cure	Below 40%	No Re-sponse	Total Cases
31	14	45	37	41	169	108	445

Trials with safoof and zamad:

The safoof and zamad formula was tried on 1300 *Bars* cases, irrespective of patients age, sex, chronicity of the disease or extension of the lesions.

The duration of illness ranged in these 1300 cases from 1 month to 30 years and above. Details shown are in Table IV.

TABLE IV

Showing the duration of illness in 1300 patients treated with Safoof and Zamad

DURATION OF ILLNESS	NO. OF PATIENTS
1-11 months	280
1-5 months	578
6-10 years	261
11-15 years	97
16-20 years	58
21-25 years	8
26-30 years & above	18
TOTAL	1,300

It was noted that in majority of the cases the duration of illness was quite long, when they attended the Institute.

The duration of treatment with safoof and zamad was from 3 to 12 months and above. Details are shown in Table V.

TABLE V

Showing the duration of treatement with safoof and zamad in 1,300 patients

DURATION OF TREATMENT	NO. OF PATIENTS
1 -3 months	290
4 -6 months	374
7 -9 months	181
10 - 12 months	187
Above 1 year	268
TOTAL	1,300

The majority of these cases received treatment for 9 to 12 months.

Response to the treatment with safoof and zamad:

Out of 1300 cases, 1094 (84.15%) responded to the given treatment and in 206 cases (14.85%) there was no reponse. The results are shown in Table VI.

TABLE VI

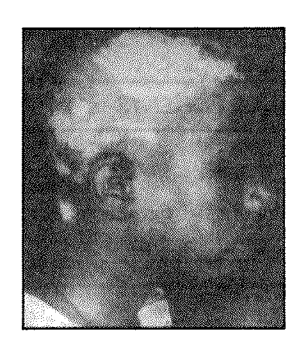
Showing the response to the treatment with Safoof and Zamad

100% Cure	91-99% Cure	71-90 % Cure	51-70% Cure	41-50% Cure	Below 40%	No Re-sponse	Total Cases
31	44	157	135	147	580	206	1,300

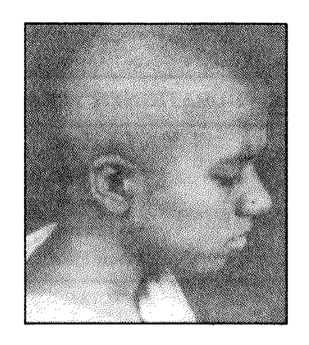
DISCUSSION

In majority of cases the depigmented patches turned red after 2 or 3 application of zamad and when continued the repigmentation started within 2 to 8 weeks. *Bars* patches found on chest, arms, face, forehead, scalp, back, neck and legs responded well to the treatment given. The patches found on back of the hands, feet and above the iliac crest showed slow reponse to the treatment.

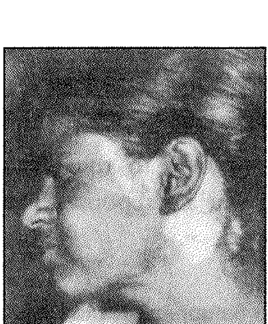
PHOTOS SHOWING THE EFFECT OF ZAMAD TREATMENT ONLY



Before Treatment 10.3.78



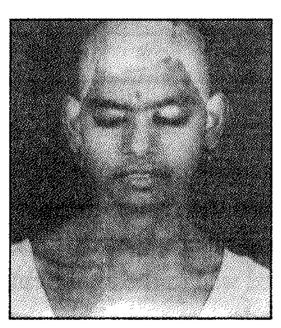
After Tratment 26.7.78



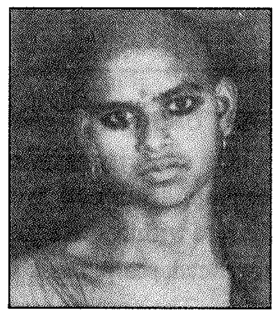
Before Treatment 27.7.76



After Treatment 17.1.78

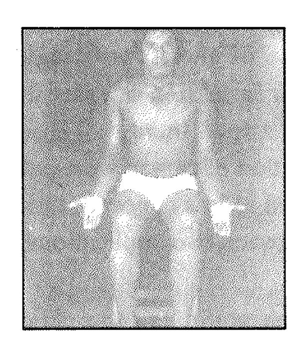


Before Treatment 28.5.77

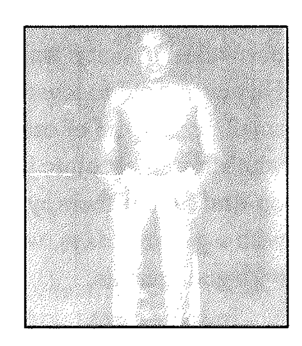


After Treatment 23.5.78

PHOTOS SHOWING THE EFFECT OF COMBINED TREATMENT WITH ZAMAD AND SAFOOF



After Treatment 20.3.79



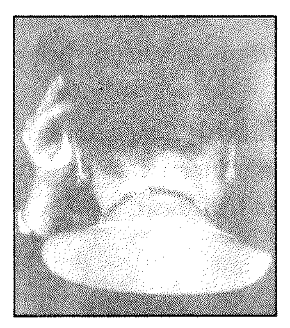
Before Treatment 28.12.77



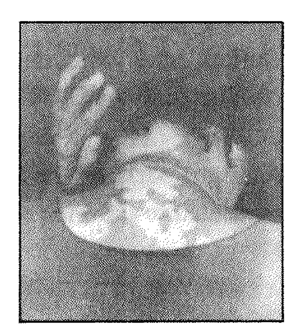
After Treatment 11.1.78



Before Treatment 14.6.77



After Treatment 11.1.78



Before Treatment 14.6.77

Safoof and zamad therapy was found more effective compared to zamad alone, and was well tolerated by majority of patients who gave maximum response to the treatment within 9 to 12 months. No particular toxicity was noted with the use of these two drugs. However, mild to severe erythema and blister formation was seen in small percentage of cases (below 10%) with zamad therapy. Those who showed absolute intolerance to application of zamad were put on oral safoof therapy. In others it was tried in less concentration with a gap of 3 days, week or a fortnight between two applications, with good results.

In some cases taking safoof orally, nausea, vomiting and gastric discomfort were reported which were controlled either by adding pure ghee السمن الخالص in diet or discontinuing the drug for few days. Urticarial rash was also seen in some cases. These side effects were not much severe and were controlled by adjusting the dosage and rarely with anti-allergic drugs.

The follow-up study of the patients was done for 1 to 2 years. Recurrences were noted in negligible number of cases. Recurrences were less in cases of complete cure and more in incompletely recovered. It can be thus concluded that safoof and zamad therapy is quite effective in the treatment of *Bars* (Vitiligo), comparing the results obtained by other disciplines. These two drugs, safoof and zamad, are taken as standard reference drugs for double blind clinical trials with new coded drugs for *Bars* which are being conducted in the CRIUM, Hyderabad.

Some of the photographs of the patients having *Bars* lesions on different parts of the body taken before and after treatment are presented to show the cure and the duration of treatment.

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THERAPEUTIC RESPONSE OF ARAB MEDICINES IN CASES OF LAQUWA

Nazeer Ahmed Siddiqui and Mohd. Zahoorul Hasan

INDIA

THERAPEUTIC RESPONSE OF ARAB MEDICINES IN CASES OF LAQUWA*

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INDIA

Abstract:

25 cases of Laquwa were treated during the Post Graduation studies at Hyderabad, India, in 1975 -1976 by simple polypharmaceutical recipe of (1) Hebbe-e-Mafasil Faliji and (2) Ustukhuddoos (*Lavandula stoechas*). The main ingredient of Habb-e-Mafassil Faliji is *Commiphora mukul* (Muquil or Gugulu) having the ratio 2:1 in the formula. All the drugs in the specific formula are anti-inflammatory, resolvent and anti-suppurative. This Habb was supplemented by the decoction of *Lavandula stoechas*, 6 grams in a glass of water. This complex treatment proved very effective as 76% cases got complete recovery in the maximum duration of 21-80 days.

INTRODUCTION

Laquwa is a name of an eagle (Uqaab). Abu Ubaidah presented his opinion that the name of 'Laquwa' has been assigned to this disease, as the patient, whenever his face is paralysed, has similar type of wider angles of his mouth, as the bird possesses. Others have suggested that Uqaab always keeps his head deviated to one side, therefore this resemblance is appropriate to name the disease so[19].

Sir Charles Bell (1774-1842) first published his idea of new anatomy of the brain in 1807. In 1823 he described the Bell's phenomenon, nearly always present in the patients of Laquwa. His

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description and research was so complete that the disease has been named after him as Bell's palsy[11].

From A.H. Rabban Tabri[33] to Avicenna[1], every author had shown the phlegm or cold moist viscid serous humour, as one of the cause for producing the disease of laquwa. Avicenna[1] says (1) that some time laquwa was caused by the spasm which occurred at one side of the face and this spasm was due to the Yaboosat or dryness. The translator of the book "Sharh-e-Asbad" confirmed his opinion, (2) some times the spasm was due to the cold and viscid humour coming from the brain and filling the nerves of a jaw, which caused the sapsmodic side to drag the healthy (other) side towards itself. By this the fairness of the lips and eyes which could be closed evenly and properly was lost from the healthy side and (3) another factor was that the angle of the mouth drooped[18, 19, 28].

Hakim Azam Khan[21] mentioned that the disease was caused by the spasm or paralysis of the muscles of the face and eye lashes. For this reason it has been named accordingly i.e. Laquwa-e-Isterkhai (Flaccid) and Laquwa-e-Tashannujee (spasmodic) or spastic paralysis[29].

Galen ascertained that the disease was due to the indifference of the matter (morbid matter) and the infiltration of viscid matter, which caused spasmodic condition at one side, while the fluid matter produced flaccidity on the other side. Usually, Laquwa-e-Tashannuji (Spasmodic) was predominent. The other type Isterkhai (flaccid) was rare [28, 29].

The pathogenesis of Bell's palsy is still not known. It is thought to be due to compression of the nerve fibres from acute inflammation and oedema of the collagenous and elastic tissues in and around the nerve. The nerve may later be reduced to only a fibrous cord[10,34].

Bell's palsy is a paralysis of the muscles of one side of the face, sometimes precipitated by exposure, chill or trauma[24].

Although it is said that 'Laquwa' is due to cold and viscid phelgm and have the influence of cold climate and season and is a disease frequently met within cold countries, but a fairly good number of cases are found in India and had been treated in different ways from centuries.

The purpose for the selection of this topic at the postgraduation studies was that, no special attention has been paid in the medical field upon this oldest known yet most neglected disease up to this time. Hitherto the different types of single and compound drugs mentioned by the ancient authors are being used without keeping in view the definite criteria.

Though in many cases of 'Laquwa' complete recovery occurs after a month or so, but if at the end of 3 weeks from the onset, there is no return of any voluntary power in the face, the recovery is never complete and contractutre usually develops later in the paralysed muscles.

The aim of the present study was to evaluate the benefits with drug therapy and to assess the efficacy of the selected specific medicines chosen for the trial on the patients of Laquwa.

MATERIALS AND METHODS

(A) ***Patients:*** 25 cases of Laquwa were selected during the studies in 1975-76 at Hyderabad, India. No particular principle has been followed in selection of cases. Besides the aetiological factors, the response of drugs especially selected for the treatment of this disease was carefully noted. The cases were investigated and followed up.

(B) ***Medicines used***: After intensive study of the ancient compound recipes, the following drugs were selected :- (1) Habbe-e-Mafasil

Faliji and (2) The decoction of "Ustukhuddoos" (*Lavandula stoechas*).

The ingredients of "Habb-e-Mafasil Faliji" were as follow:

(1) Dar-e-filfil - (root)/ *Piper longum*

(2) Zanjabeel (root)/ *Zingiber officinalis*

(3) Kababa (dried fruits) / *Cubeba officinalis*

(4) Zarambad/*Curcuma zerumbet*

(5) Abhal (Berries)/ *Juniperis fructus communis*

(6) Filfilmoya/*Piper longum* or *Chavica roxburgii*

(7) Kasni/ *cichorium intybus*

(8) Podina/ *Mentha arvensis*

(9) Muqul/*Balsamodendron mukul* or *Commiphora mukul* (Gugal) (Burseraceae) *Hooker Stedor*[16].

(10)Rooghan-e-Kunjad/ *Sesamum indicum* (Oil) for mixing the powder.

The ratio of 'Muqul' and all other drugs in the above selected formula was 2:1, so the main drug of this recipe was Muqul (*Balsamondendron mukul* or *Commiphora mukul*).

The ancient authors have described Mukul as Mohallil (Resolvent), Mushil-e-Balgham (Purgative of Phlegm) and have mentioned that it dries the Rutoobat also[22, 29]. It is useful when used in phlegmatic disorders such as paralysis, facial paralysis etc[27]. It eliminates abnormal fluids and acts as anti-inflammatory[7, 15, 17, 20, 22].

K.M. Nadkarni[26] has mentioned the Mukul's action as demulcent, aperient, carminative, antispasmodic and emmenagogue. He also mentions that "Gugul" (Mukul) is said to have marked antisuppurative properties and it causes an increase of leucocytes in the blood and hence stimulates phagocytosis. Chopra[9] mentions that it is quite harmless and may be taken for a long time without any side effects.

Recent pharmacological and clinical studies on the oleoresin portion of the plant have found it to be a highly potent anti-inflammatory agent as hydrocortisone[13]. The anti-arthritic and anti-inflammatory activities were also studied by some more scientists afterwards and found that oleoresin fraction possessed significantly active antiarthritic and anti-inflammatory properties and the antiphlogistic effect was comparable to that of hydrocortisone acetate[4,16,31,32].

The other drugs contained in this formula i.e. Filfil moya (root of *Piper longum*), Abhal (*Juniperis fructus communis*), Zarambad (*Curcuma zerumbet*) and Kababa (*Cubeba officinalis*) possess the properties of anti-inflammatory and antispasmodic and resolvent agents and Podina (*Mentha arvensis*), Kasni (*Cichorium intybus*) and Zanjbil (*Zingiber officinalis*) were carminatives, stimulants and digestives. Therefore, most of the drugs were anti-inflammatories, stimulants, resolvents, carminatives, antispasmodics, in action, while Abhal (*Juniperis fructus communis*) was a vasodilator.

Another drug which was selected for the trial was Ustukhuddoos (*Lavandula stoechas*). Its numerous actions were known to the ancient physicians, such as stimulant, aromatic, carminative, antispasmodic, antiphlogistic and resolvent and deobstruent[9, 20, 26].

PREPARATION OF DRUG:

All the crude drugs of this recipe were powdered separately except Mukul. As Mukul was sticky so first of all, it was mixed with sesame oil and then other ingredients of the formula were added. The semi-solid compound so formed was made into tablets of 2 gms and then given to the patients.

DOSAGE:

Generally Habb-e-Mafasil Faliji was prescribed in divided doses according to age and sex. It was given from 4-6 grams in 24 hours.

ADMINISTRATION OF THE MEDICINES:

The main drug Habb-e- Mafasil Faliji was administered to the patients of Laquwa in the divided doses of 4-6 grams in 24 hours. The decoction of Ustukhuddoos (*Lavandula stoechas*) 6 grams in a glass of water was also used with it. Some of the patients were kept on Habb-e-Mafasil only without giving decoction, just to assess the efficacy of the main drug.

DISCUSSION ON TREATMENT

There is no specific treatment for facial paralysis in any system of medicine. The reason is that the aetiological factors are still unknown. In this series a polypharmaceutical recipe has been formulated and tried on the patients of Laquwa on the basis of generalised concept of Greco-Arab system of medicine. The therapeutic response of this complex treatment was promising and was as follows:-

Cured - 76%, Relieved - 16%, Otherwise - 8%.

TABLE 1:

Showing the overall therapeutic response of drugs in cases of Laquwa

Result	No. of Patients	% of cases responded
Cured	19	76%
Relieved	4	16%
Otherwise	2	8%
Total No. of Patients	25	–

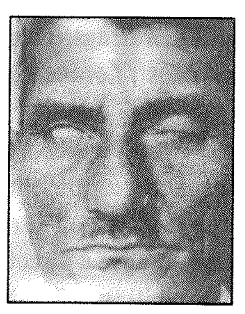
The specific medicine known as Habb-e-Mafasil Failji consisted of *Commiphora mukul* in combination with other drugs. This was supplemented by the decoction of Ustukhuddoos *(Lavandula stoechas)*.

The above medicines have been used by Greco-Arab Physicians since centuries as they were aware of their actions, and our present experience has also confirmed the same. The results of the treatment were very satisfactory. The medicines might have a direct anti-inflammatory effect on the tissues, thereby inhibiting cellular reaction, or they might exert the anti-inflammatory effect through the pituitary - adrenal axis. Cortisone might be liberated and be directly responsible for minimising inflammation at the site. So it is also quite likely that these drugs might be stimulating the pituitary to activate the adrenal function.

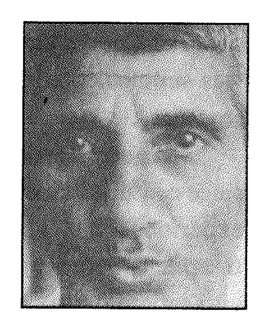
The effects of the anti-inflammatory action of prednisone were tested in 40 cases by Fayez and Ragheb of the Cairo University in 1960. It was effective in the treatment only when given very early in the disease (i.e. in the first 24 hours[12]). It is usually customary to give ACTH as soon as possible after the palsy has occurred (Fearnley, et al 1966). Adrenal corticosteroids begun near the onset of illness and continued for 7 to 10 days may favour a more rapid and complete recovery[6].

K.K. Adour and J. Wingerd[3], determined that the oral prednisone is the treatment of choice for all patients with idiopathic facial palsy.

(Sex - Male, Age - 50 years, Duration of Treatment - 25 days.)

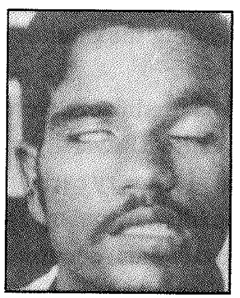


Left sided Laquwa.
At the time of admission

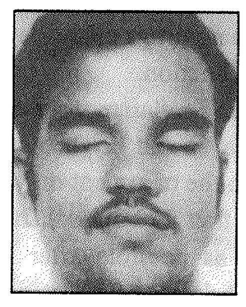


After the Treatment.

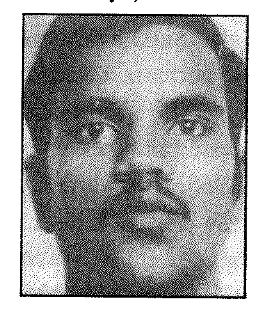
(Sex - Male, Age - 18 years, Duration of Treatment - 100 days.)



Right sided Laquwa.
A few days after the admission.

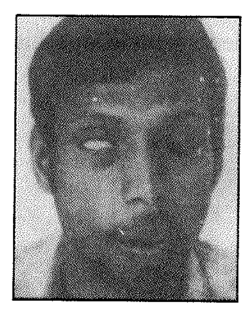


During the Treatment

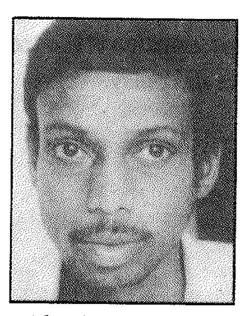


After the Treatment.

(Sex - Male, Age - 22 years, Duration of Treatment - 103 days)

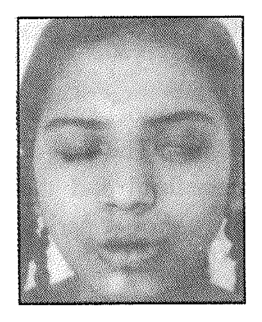


Right sided Laquwa.
Before the Treatment.

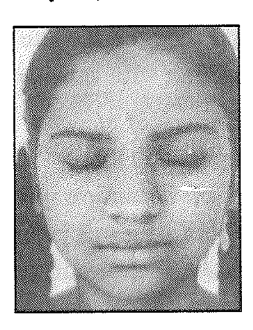


After the Treatment.

(Sex - Female, Age - 10 years, Duraton of Treatment - 27 days.)



Left sided Laquwa.
At the start of the treatment.

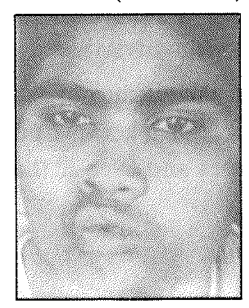


During the Treatment.

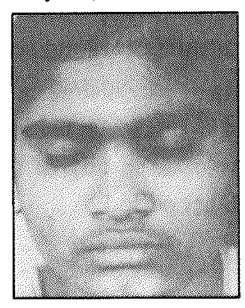


After the Treatment.

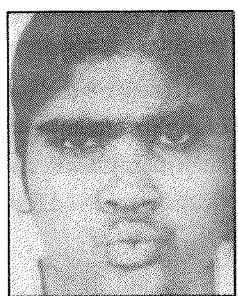
(Sex - Female, Age - 16 years, Duration of Treatment - 41 days.)



Left sided Laquwa.
A few days after admission.

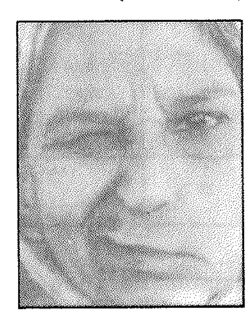


A few days before discharge.

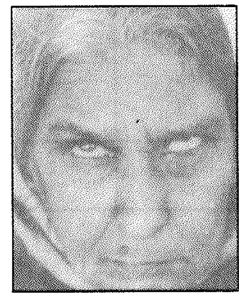


After the Treatment.

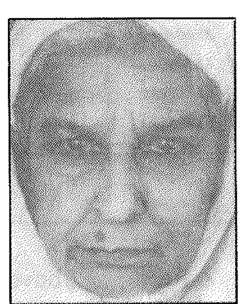
(Sex - Female, Age - 60 years, Duration of Treatment - 84 days.)



Left sided Laquwa
A few days after admission.



A few days befor discharge



After the Treatment.

The advantage of using the Mukul is its ability to penetrate any barrier in the system and to reach the site of action. Its action gets doubled when mixed together with various vehicles[27].

The second drug *Lavandula stoechas* (Ustukhuddoos) sweeps away all the phlegm impurities (Kabir[20]; Nadkarni[26]). The trial of this drug in the form of decoction proved beneficial and the therapeutic response of this drug was promising in a shorter period when given to the patients with the main drug.

Nowadays the corticosteroids having their pronounced anti-inflammatory and analgesic properties have found application in the treatment of rheumatic diseases, but the prolongation of steroid medication increases the frequency of side effects and therefore its un-interrupted administration over long periods is un-advisable[2,3].

Keeping the hazards of prednisone in view, the efficacy of the complex treatment tried in our series is most beneficial, because no such complications developed in any of the cases and even if the drugs were used for a longer period.

TABLE 2: Showing the duration of treatment and percentage recovery

Duration	Cured	Relieved	Otherwise	Total	%
0-20 days	-	-	1	1	4%
21-40 days	9	3	1	13	52%
41-60 days	5	-	-	5	20%
61-80 days	2	-	-	2	8%
81 above	3	1	-	4	16%
Total: % response	19 76%	4 16%	2 8%	25	-

From the above table it is clear that the maximum duration of 21 to 40 days was required for 52% cases and the next duration i.e. 41 to 60 days was required for 20% cases.

Out of 25 cases of Laquwa treated by the complex treatment 76% cured, 16% relieved and 8% otherwise. If the patients in the relieved groups would have continued the therapy for a longer period, they might have got complete recovery.

TABLE 3: Showing Laquwa recovery profile

SITE	Percent return of function				
	0 to 25%	25 to 50%	50 to 75%	75 to 100%	Total
Forehead	-	2	4	19	25
Eye	-	2	3	20	25
Mouth	-	3	4	18	25

In 80% patients, eye abnormalities recovered completely and in 76%, forehead wrinkles recovered completely and in 72% deviation of mouth recovered completely.

CONCLUSION

It is concluded that this complex treatment has proved very effective for cases of Laquwa because the method is simple, drugs are cheap, no complications developed even if the therapy was continued for a longer period and there was no recurrence in any case reported. The therapy was very effective as 76% recovered completely and 16% were relieved.

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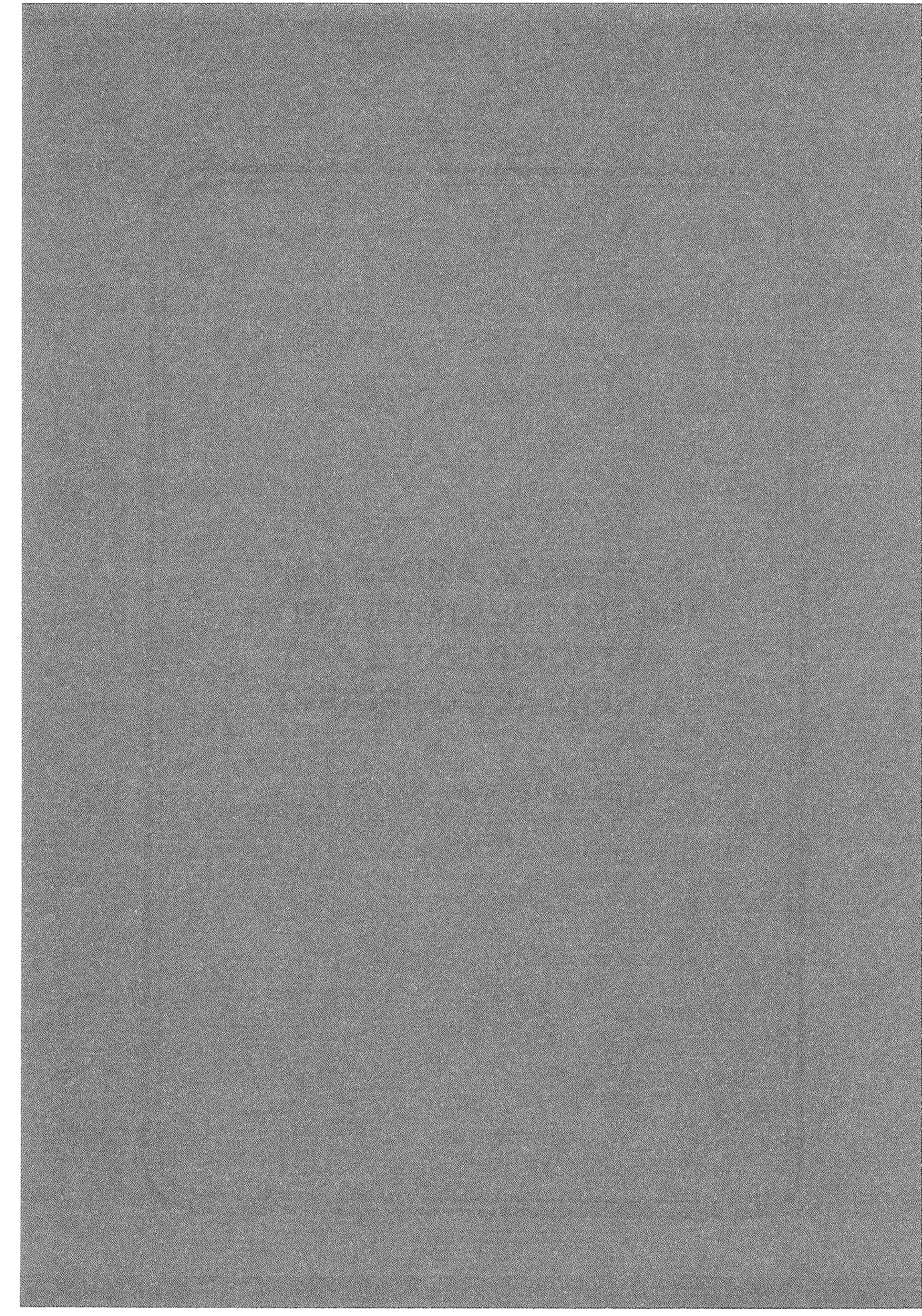
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INTESTINAL AMOEBIASIS AND ITS TREATMENT WITH METRONIDAZOLE AND A HERBAL COMPOUND

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INTRODUCTION

Amoebiasis is an endemic disease with varying intensity in many areas in the world particularly in the tropical and developing countries.

Althouth various chemical drugs are fairly effective against amoebic dysentery yet none of them is free from side effects. The iatrogenic diseases are increasing gradually all over the world.

Herbal antiamoebic medicines, on the other hand, were reported over many centuries ago until now to be fairly effective and free of side effects.

In this work, we tried to do a comparative study between the antiamoebic effect of metronidazole and certain herbal medicines used in the treatment of amoebiasis in Islamic medicine.

MATERIAL AND METHODS

The herbal medicines were prepared from the following herbal ingredients:

Name described in the text of Islamic Medicine	Botanical Name
1. HABB- UL-AS	*Myrtus communis*
2. AFAS	*Quercus infectoria*
3. MAMIRAN	*Coptis teeta*
4. BAZAR-UL-BUNJ	*Hyocyamus niger*

All of the four ingredients taken in equal quantity, were reduced to form a compound powder at Takmeel-ut-Tib Pharmacy.

* Bulletin of Islamic Medicine, 2: (473-476), 1982.

The present study was carried out at the Clinic of Takmeel-ut-Tib Pharmacy, Lucknow. Children brought with gastro-intestinal complaints along with other cases attending the clinic for some other diseases were subjected to normal routine stool examination and only those 50 cases, whose stool examination showed cysts or trophozoites of *Entamoeba histolyica* were selected for present study and these cases were subjected to detailed investigation and examination. Stool examination was done by direct smear examination and iodine staining methods.

On the basis of therapy used the cases were classified into two groups.

GROUP	NO. OF CASES	DRUG	DOSE/KG	DURATION
H	25	Trial drug	T.D.S (50mg)	7 days
M	25	Metronidazole	T.D.S (50mg)	7 days

Stoool examination of both groups 'H' (herbal compourd i.e. the test drug) and 'M' (metronidazole) was done on 8th, 9th and 10th day. The case that had become negative for *Entamoeba histolyica* on three consecutive days was again subjected for stool examination on the 17th day to reconfirm the reappearance or disappearance of the cysts and trophozoites.

RESULTS

TABLE I

AGE IN YEARS	TOTAL NO. OF CASES	GROUP 'H'	GROUP 'M'
		NO. OF CASES	NO. OF CASES
1-2	3	1	2
3-4	5	3	2
5-6	10	4	6
7-8	8	2	6
9-10	6	5	1
11-12	4	2	2
13-14	14	8	6

TABLE II

SEX INCIDENCE IN *ENTAMOEBA HISTOLYTICA* INFESTED CHILDREN

SEX	TOTAL NO. OF CASES	GROUP 'H'	GROUP 'M'
		NO. OF CASES	NO. OF CASES
MALE	35	18	17
FEMALE	15	7	8

TABLE III

SHOWING THE PRESENCE OF *GIARDIA LAMBLIA* IN PATIENTS WITH *ENTAMOEBA HISTOLYTICA*

ASSOCIATED PARASITES	TOTAL CASES	GROUP 'H'	GROUP 'M'
	NO. OF CASES	NO. OF CASES	NO. OF CASES
E. histolytica Alone	18	9	9
E. histolytica with *Giardia*	14	7	7

TABLE IV

SHOWING NUMBER OF SYMPTOMATIC CASES IN *ENTAMOEBA HISTOLYTICA* INFESTED CHILDREN

SYMPTOMS	TOTAL CASES NO. OF CASES	GROUP 'H'	GROUP 'M'
		NO. OF CASES	NO. OF CASES
Symptomatic	36	16	20
Asymptomic	14	9	3

SIDE EFFECTS

In group 'H', one case had complained of loose motion and one reported mild abdominal discomfort, which were considered due to the disease process rather than due to the medicine. No effects were detected on white cells or on platelets.

In group 'M', one case had several complaints like headache, pain in abdomen, nausea, vertigo and bitter taste and the drug was discontinued on 3rd day. Two other cases had nausea and headache and one had marked flatulence.

TABLE V

SHOWING RESULTS OF TREATMENT IN BOTH GROUPS OF *ENTAMOEBA HISTOLYTICA* INFESTED CHILDREN

RESULT	GROUP 'H'	GROUP 'M'
	NO. OF CASES	NO. OF CASES
Cured	21	22
No response	4	3

TABLE VI

SHOWING EFFECT OF BOTH DRUGS ON *GIARDIA*

WORM	GROUP 'H'		GROUP 'M'	
	INITIAL NO.	DISAPPERANCE	INITIAL NO.	DISAPPEARANCE
Giardia	7	3	7	5

DISCUSSION

Fifty cases of intestinal amoebiasis were included in the present study and were equally divided into two groups-H (given Herbal medicine) and M (given Metronidazole). The duration of treatment in both groups was the same. The clinical improvement was judged by the recovery from the symptoms as well as disappearance or absense of cysts and trophozoites in the stool.

28% of the patients were in the age group of 13 to 14 years and 36% of the patients were under 10 years of age (Tables I and II).

Stool examination demonstrated 36% cases of *Entamoeba* alone. *Giardia lamblia*, were detected in 28% of cases (Table III). Asymptomatic cyst passers were also detected (Table IV).

After one week therapy the clinical cure in the cases of group 'M' who received metronidazole, was 88% as compared with the cases of group 'H' who were on the herbal medicine (84%) (Table VI). It was observed that the 'Herbal medicine' has antigiardial property also (Table VI).

In group 'H', no side effects were noted while in group 'M' who received Metronidazole three patients had side effects such as headache, vertigo, nausea and flatulence and one case discontinued the treatment on the third day.

From this study it was concluded that though metronidazole used for intestinal amoebiasis has a better response but the hazards of side effects exist with it. While, on the other hand, the herbal medicines have a fairly good response (84%) without any side effects.

Owing to the danger of widespread iatrogenic diseases due to chemical preparations in treatment, we have to search for effective non-toxic and potent preparations for various ailments out of the texts of the classics of Islamic Medicine.

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